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RESEARCH ARTICLE

RICE – *RHIZOBIUM* INTERACTIONS FOR BIOLOGICAL NITROGEN FIXATION: TECHNICAL CHALLENGES

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Abstract: Nitrogen is the most important nutrient input required for rice production. As most of the soil is deficient of N, N-fertilizers are needed. But, instead of chemical fertilisers, biological nitrogen fixation (BNF) is preferred. In that too, conventional BNF has limited capacity to render rice independent of external sources of N. Therefore, a major goal of BNF research has been to extend the nitrogen fixing capacity to rice. In this context, recent advances in understanding symbiotic *Rhizobium*-legume interactions at the molecular level, the discovery of natural endophytic interactions of rhizobacteria with rice, potentiality of rice nodulation, as well as potentiality of introduction / expression of *nif* genes in (to) rice has offered exciting opportunities to stretch rice research horizons, though there are technological challenges. These aspects have been reviewed in this article.

Keywords: Rice-*Rhizobium* interactions, Biological nitrogen fixation, Endophytic association, Nodulation in rice

INTRODUCTION

Soil bacteria are very important in biogeochemical cycles. Among them, certain bacteria exert beneficial effects on plant development, which are defined as plant growth-promoting rhizobacteria (PGPR). They exert beneficial effects via direct and indirect mechanisms. The direct mechanisms include nitrogen fixation, production of hormones, production of enzymes and mobilization of nutrients (Khalid *et al*, 2004; Lucy *et al.*, 2004; Gray and Smith, 2005). The indirect mechanisms include increasing efficiency of fertilizers uptake, increasing the plant's tolerance towards stress, inducing host resistance or producing pathogen-suppressing substances (Raj *et al.*, 2003; Van Loon, 2007). These can work independently or simultaneously with each other (Keyeo *et al.*, 2011). The said PGPR can be divided into two groups, *viz.*, symbiotic bacteria and free-living associative bacteria considering their relationship with the plants (Khan, 2005), as well as according to their residing sites (Gray and Smith, 2005). The symbiotic bacteria live inside the plant cells in specialised structure forming nodules and free-living rhizobacteria live outside the plant cells and do not produce nodule, but still promote plant growth.

Among direct mechanisms, nitrogen fixation is considered to be most important, in which inert N_2 is converted to NH_3, a form that plants can use. As the conversion is by and in living organisms, the process is defined as biological nitrogen fixation (BNF). Again, BNF by symbiotic system has got advantage over associative nitrogen fixation. In associative nitrogen fixation, the involved bacteria in the rhizosphere of plants utilise the products of nitrogen fixation for their own growth, but release little while they are alive (Van Berkum and Bohlool, 1980). On measurement of associative nitrogen fixation using ^{15}N in rice, as well as in wheat, Okon (1985) confirmed that, the majority of the fixed nitrogen remain in the bacteria within the root environment. On the other hand, in symbiotic systems, fixed nitrogen becomes directly available to the plants due to more intimate metabolic exchange with host plants. Hence, the nitrogen-fixing bacteria colonize the plant internally and become endophytic, thus, insulated from competition with other rhizosphere microorganisms too (Reddy *et al.*, 1997; Webster *et al.*, 1997).

But, symbiotic system is confined in leguminous plants, and the best known symbiotic bacteria belongs to the genera *Rhizobium* (Hayat *et al.*, 2010). For BNF, the non-leguminous plants including cereal plants such as rice, there is dependency on free-living nitrogen-fixing bacteria. However, several researchers observed natural association of rice with rhizobia and also reported certain interactions in laboratory as well as in field experiments (Yanni *et al.*, 1997). As a result, possibilities of enhancement of natural association of rice with rhizobia (Yanni *et al.*, 1997) and even to the extent of extension of nodulation characteristics (Reddy *et al.*,1997) have been envisaged. Development of biotechnological tools have also generated optimism of incorporation / expression of nitrogen fixing genes in (to) rice (Stoltzfus *et al.*, 1997). However, inspite of potentialities, there are certain technological challenges.

Rhizobium : the obligate symbionts in leguminous plants

Rhizobia were first classified on the basis of cross-inoculation capability, *i.e.*, the ability to nodulate a specific group of hosts (Fred *et al.*, 1932). The seven cross-inoculation groups identified were :

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Sinorhizobium meliloti which nodulate alfalfa group, *Rhizobium trifolii* which nodulate clover group, *Rhizobium legumenosarum* which nodulate pea, *Rhizobium phaseoli* which nodulate bean, *Rhizobium lupine* which nodulate lupine group, *Bradyrhizobium japonicum* which nodulate soyabean and *Rhizobium* sp. which nodule cowpea (Fred *et al.*,1932). But, this method most variably failed in classifying different species (Coutinho *et al.,* 2000) as classification of rhizobia on the basis of host range and physiological properties does not reflect the true phylogeny of the group (Sahgal and Johri, 2003). Later molecular systematic provided a breakthrough and helped to evaluate *Rhizobia* diversity in different environments (Patel and Sinha, 2011). The present taxonomy of *Rhizobia* comprises of 98 species found in 13 genera (Weir, 2012). Most of these bacterial species are in genera either *Rhizobium*, *Mesorhizobium*, *Ensifer* and *Bradysrhizobium* (Annapurna and Govindasamy, 2009). Among them, the best known PGPR is the genera *Rhizobium*, unlike many other soil microorganisms, produce no spores and are aerobic and mobile (Baset Mia and Shamsuddin, 2010). Though, they live freely in the soil in the root region of both leguminous and non-leguminous plants, can enter into symbiosis only with leguminous plants (Subha Rao,1982; Bottomley and Dughri,1989; Bottomley and Maggard,1990).

In symbiotic association with legume, the *Rhizobium* form morphologically defined structure called nodules by responding chemotactically to flavonoid molecules released as signals by the legume host. These plant compounds induce the expression of nodulation (nod) genes, which in turn produce lipo-chitooligosaccharide signals that trigger mitotic cell division in roots, leading to nodule formation (Dakora,1995; Lhuissier *et al.,* 2001; Matiru and Dakora, 2004).

The nodulated legumes contribute a good deal to the amount of nitrogen fixed in the biosphere. BNF is estimated to contribute 180 X 106 metric tonnes per year globally (Postgate, 1998), of which 80 per cent comes from symbiotic associations and the rest from free-living or associative systems (Graham,1988). However, although, the value of cultivating legumes for BNF was known through centuries, experimental evidence in support of it came only in the later half of the nineteenth century from the results of a classical experiment conducted by Boussingault in 1883 (Fred *et al.*,1932). In another classical experiment, Wilforth and Hellriegal in 1888 compared the performance of oats and peas and concluded that, the root nodules are responsible for the special ability of pea plants to use atmospheric nitrogen (Fred *et al.,* 1932). Hellriegal and Wilfarth in 1888 also demonstrated that an increase in nitrogen content in soil is due to the presence of small tumer-like outgrowth on the roots of leguminous plants (Sharma, 2003). It was also found that, in absence of root nodules, growth of succeeding plant group was retarded. The results of various experiments revealed that, root nodule forming plant can feed upon atmospheric nitrogen and this capacity of plants can be observed only in those plants, which are growing in non-sterilized or bacteria containing soils (Sharma, 2003). These symbiotic bacteria utilize the free atmospheric nitrogen and synthesize it into new nitrogenous compounds, which are utilized by plants for their growth; and bacteria get their food from these plants. Such a mutual beneficial association of bacteria and plants is referred to symbiosis.

Natural association between *Rhizobium* and rice

In nature, *Rhizobium* is normally viewed as a microbe that survives saprophytically in soil between periods in which the host legume is absent. However, studies in Egypt have shown that clover (*Trifolium alexandrinum*) rhizobia also occupy another endophytic niche inside rice plant. Yanni *et al.* (1997) isolated *Rhizobium leguminosarum* bv. *trifolii* as a natural endophyte from roots of rice in the Nile delta, where rice has been grown in rotation with berseem clover (*Trifolium alexandrinum*) for about seven centuries. This probably promoted closer rhizobial affinity to this cereal as a 'host plant'. This hypothesis is re-enforced by the fact that population of clover-nodulating *Rhizobium* isolated from rice could occur up to 2.5 x 10^7 cell g^{-1} fresh weight of root, concentrations similar to those obtained for bacteroids in legume root nodules (Hayat *et al.,* 2010), and have potential to promote rice growth and productivity (Yanni *et al.*,1997).

From Senegal and Guinea, Chaintreuil *et al.* (2000) similarly isolated photosynthetic *Bradyrhizobia* from roots of *Oryza breviligulata*, an ancestor of African brown rice *Oryza glaberrima*, which generally grows in the same wetland where *Aeschymene sensitiva* (a stem-nodulated legume, associated with photosynthetic strains of *Bradyrhizobium*) grows. This suggest co-evolution of *Aeschynomene*, *Bradyrhizobia* and wild genotype of African brown rice (*Oryza glaberrima*), though whether the *Bradyrhizobia* affect growth of *Oryza glaberrima* plant has not been determined (Hayat *et al.,* 2010). Natural associations of endophytic diazotrophs in rice roots under rice-*Sesbania* rotation in the Phillipines has also been reported (Ladha *et al.,* 1989, 1996;Yanni *et al.*,1997). In Nepal too, endophytic bacteria of native races of rice *azorhizobia* have been reported (Engelhard *et al.,* 2000), but there was no report on clear association of the rhizobial endophytes with legumes.

Above evidences suggest that, there may be natural endophytic association between *Rhizobium* and rice, as well as stimulation of plant growth, but there is no conclusive evidence that the benefits involves symbiotic nitrogen fixation (James, 2000;Yanni *et al.,* 2001). Though there is no conclusive evidence, if it happens, there is question, how efficiently the

endophytes can actually function for nitrogen fixation, when no obvious 'symbiotic' structures appear to be present.

Rhizobium as bioinoculum in rice cultivation

From time immemorial rhizobia have been used as bioinoculants for increasing the yield of legume crops. Recent trials shown that, it also play role in enhancement of production of rice. In field inoculation experiment, Yanni *et al.* (1997) observed that, two rice endophytes *Rhizobium leguminosarum* bv. *trifolii* E11 and E12 significantly increased shoot and root growth, as well as grain yield by 46 and 42 per cent respectively. Under green house conditions, a 20 per cent increase in shoot growth and grain yield of the wild rice *Oryza breviligulata* was obtained by inoculation with photosynthetic endophytic *bradyrhizobia* (Chaintreuil *et al.,* 2000). Yanni and Dazzo (2010) has conducted large-scale field experiments in Egypt and evaluated 5 rice (*Oryza sativa*) varieties inoculated with 7 endophytic rhizobial strains during 5 growing seasons, including at sites ranked as the world's highest in rice production. Inoculation with single strains or multi-strain consortia significantly increased grain yield in 19 of the 24 trials. By combining superior rhizobial inoculants with agricultural extension training, grain yield increased up to 47 per cent in farmers field, with an average increase of 19.5 per cent. Data on rice straw production, harvested index and the agronomic fertilizer N-use efficiency also indicated positive agronomic benefits of rhizobial inoculation. Studies conducted at the IRRI too showed that its inoculation increased growth and yield of rice, and N, P and K uptake by rice plants significantly (Biswas *et al.,* 2000, 2000a). Dey and Srivastava (2001) opined that, the *Rhizobium* can fix N in the soil as free-living nitrogen fixer and there are reports that, *Rhizobium* utilizes the N from soil by promoting physiological growth response generating changes to the root morphology of the rice plant that favours its uptake (Biswas *et al.,* 2000; Yanni *et al.,* 2001;Kennedy *et al.,* 2004; Askary *et al.,* 2009).

Present authors also observed enhanced growth, enhanced metabolic and enzymatic activity in two high yielding varieties of rice plants (cv. IR – 64 and cv. NDR – 359 of *Oryza sativa* L.) in paddy field in West Tripura, India (Roy and Srivastava, 2010; Roy, 2013). For basal inoculation of rice plants, the *Rhizobium* spp. was isolated from the root nodules of *Mimosa pudica*, a local herbaceous weed. While positive response in regards to growth, soluble protein and *in vivo* nitrate reductase (NR) activity have been observed in both the cultivars, inoculation result found to be even better than *Azospirillum brasilense*, *Azotobacter chroococcum*, *Clostridium pasteurianum* and *Pseudomonous denitrificans* in IR – 64.

Experimental trials on forced nodulation of rice

Though the molecular and cell biology of the *Rhizobium*-rice association differs in many respects from the biology underlying the development of root nodules in the *Rhizobium*-legume symbiosis (Reddy *et al.,* 1997), due to the findings of natural endophytic association between *Rhizobium* and rice, and positive result on inoculation on rice plants as mentioned above, it has been felt that 'functional *Rhizobium*-rice association' could be enhanced or created through forced interactions. In this context, several researchers have made experimental trials and reported a variety of responses, such as the ability of rhizobia to attach to rice roots (Terouchi and Syono,1990), elicit the deformation of root hairs (Plazinski *et al.,*1985), and to form nodule-like structures / hypertrophies (All-Mallah *et al.,*1989; Bender *et al.,* 1990; de Bruijn *et al.,*1995; Jing *et al.,*1990,1992; Li *et al.,*1991; Rolfe and Bender,1990) or development of thick short lateral roots on rice plants (Cocking *et al.,*1993). Though, the nodulation or nodule-like structure of rice by rhizobia has been reported, it either occurred at very low frequency (Bender *et al.,* 1990) or required an enzymatic pre-treatment of the roots (Al-Mallah *et al.,* 1989) and nitrogen fixation reported by only one group (Jing *et al.,*1990), in that case too, adequate controls were not presented (Spaink and Lugetenberg,1992). It also appears that, the nodules on rice roots (Bender *et al.,* 1990; Cocking *et al.,* 1990) does not possess highly organised internal structure of legume nodules, and hence, will not necessary provide the anaerobic environment essential for nitrogenase activity (Schell *et al.,* 1992) which possibly explain why the amounts of N_2 fixed are very low (Bender *et al.,* 1990; Cocking *et al.,* 1990; Jing *et al.,*1990). According to Reddy *et al.* (1997) it is unlikely that, a monocot plant such as rice would possess the complete complement of genes involved in the nodule ontogeny programme, and the rhizobial strains could induce the formation of genuine nodules on these plants.

Need of genetic modifications of rice plants

Interestingly, rice appears capable of perceiving Nod factors coded for by bacterial *nod* genes, and several homologues to legume *ENOD*s are present in rice (Reddy *et al.,* 1997). These (nodulin) genes specifically expressed in legumes during early events in infection and nodule formation (Reddy *et al.,* 1996; 1996a). Moreover, the promoter activity of rice *ENOD40* in soybean revealed that, its tissue-specific expression was identical to that of the endogenous soybean promoter, indicating that key regulatory features of these genes may be conserved in rice (Reddy *et al.* 2000). These results suggest that, rice may have at least part of the genetic program involved in a functional symbiosis with *Rhizobium*.

Rice also possesses the capacity to form symbiotic (mycorrhizal) associations with fungi (Secilia and

Bagyaraj, 1992; Khan and Belik, 1995). Genetic links between the processes involved in nodulation and arbuscular mycorrhiza have also been found in legumes (Gianinazzi-Pearson, 1996; Cook *et al.*, 1997). Thus Reddy *et al.* (1997) opined that, rice may possess part of the genetic programme necessary for entering into mutually beneficial, endosymbiotic associations with other soil microorganisms. Therefore, according to him, the rice would need to be genetically modified to respond to the appropriate rhizobial morphogenic triggers and subsequent *Rhizobium*-modulated nodule ontogeny requirement.

Technical options and challenges

There are three options to enhance / create 'functional BNF' in rice plants. These are : (i) enhancement of natural endophytic association of *Rhizobium* and rice (Yanni *et al.*,1997); (ii) development of novel symbiotic interactions resulting in the formation of nitrogen forming nodules or nodule like structures on rice root (Spaink and Lugtenberg,1992); and, (iii) direct incorporation / expression of the required complement of nitrogen fixation genes in (to) rice (de Bruijn *et al.*,1995; Ladha and Peoples,1995; Ladha *et al.*,1997; Dixon *et al.*, 2000; Sofi and Wani, 2007).

(i) Enhancement of natural endophytic association: The term 'endophytic' is used with various meanings in the literature on plant-microbe interactions (Saikia and Jain, 2007). Here, the term 'endophyte' is used to describe microbes that have colonized living plant tissue, but no formation of nodules and / or no harm to the host.

Existence of natural endophytic association of *Rhizobium* and rice (Ladha *et al.*,1989,1996;Yanni *et al.*,1997;Chaintreuil *et al.*, 2000; Engelhard *et al.*, 2000; Hayat *et al.*, 2010) suggest that, natural evolution itself have done some work for us, which, we can bank on. Therefore, Saikia and Jain (2007) advocated, it is advisable to observe and learn the natural phenomenon and interpret the finding in the laboratory.

Stoltzfus *et al.* (1997) also made suggestion that, first some basic knowledge about the presence, predominance and stability of endophytic bacteria in different rice tissues be obtained. The objectives of such endeavour be (i) isolating putative endophytic bacteria from diverse rice varieties grown in different soil types and assessing their diversity, (ii) developing molecular probes for the detection of putative N_2-fixing endophytes, and, (iii) studying the internal colonization of rice tissue by putative endophytic bacteria.

Saikia and Jain (2007) further opined that, the study can not be confined only to such a narrow space as the relationship between a certain host plant and a certain single strain microbe. Relationship of a plant with other plants and microbes, of the microbes with microbes and organisms, and sunlight, air, moisture, etc. are factors to be considered in the study (Saikia and Jain, 2007), which sometimes can even become crucial deciding factors under certain circumstances (G-Laboratory, 2002).

(ii) Search for nodulation in rice: Does nodulation occur naturally in rice? In investigating the feasibility of forcing nodulation in rice, it would be prudent to ascertain if nodulation occurs naturally in rice but at such a low frequency or under such unusual conditions that, it has so far escaped detection in the field (Bennett and Ladha, 1992). Bennett and Ladha (1992) also postulated that, if this is correct, then our efforts should be directed towards designing a rational search for the phenomenon and then attempting to increase its frequency through genetics and management.

But, the question is, how should we proceed? Bennet and Ladha (1992) proposed three approaches. These are: (i) to examine the roots of land races and wild species growing under N-limiting conditions, where nodulation would be an advantage and where the amount of mineralized N would be insufficient to repress nodulation, (ii) to examine the capacity of rice to support nodulation by rhizobia and other micro-symbionts derived from other plant studies, and (iii) to examine the rice genome itself for the presence of genes required for N_2-fixing symbiosis. If searches results in sufficient knowledge to design strategies for efficient nodulation and perhaps also N_2 fixation in rice, would it work in the field? This is not an unreasonable doubt as it has been observed that (i) often the rhizobia modified for superior performance in laboratory or in green house fail to establish themselves in nature (Bennet and Ladha, 1992), and (ii) the problems of achieving high levels of nodulation and N_2-fixation in legumes themselves (Keysar and Li, 1992).

Further, Saikia and Jain (2007) postulated that, no matter whether on leguminous plants or non-leguminous plants, root nodules once formed, may be either effective or ineffective, this depends on: (i) whether or not the microbes living inside the nodules are capable of fixing N; (ii) whether or not the microbes and the host plant can form a highly harmonious symbiotic N-fixing system; and (iii) the environmental condition in which the host plant is growing.

Another concern aroused by the idea of nodulation in rice relates to competition between nodules and the rest of the plant for photosynthate (Bennette and Ladha, 1992).

(iii) Incorporation / Expression of N_2-fixation genes in rice: The possibility of directly engineering nitrogen fixation in rice lies in the successful incorporation of the essential *nif* genes for nitrogenase activity into the rice genome (Britto and Kronzucker, 2004). But, the question is which is the appropriate location, where *nif* genes to be introduced? Parakaran (1997) proposed two approaches, *viz.*, transformation of rice leaf and

transformation of rice root. Merrick and Dixon (1984) and Dixon *et al.* (1997) suggested that, root plastids or chloroplasts be the most suitable intracellular locations for foreign *nif* genes, rather than cell nucleus. This hypothesis is based on the fact that, plastid genetics most closely resemble that of N_2-fixing prokaryotes (Whitfeld and Bottomley, 1983).

According to Fischer (2000), the components of action for directly engineering nitrogen fixation in rice also includes protection of nitrogenase from inactivation by oxygen and to ensure energy supply for nitrogen fixation without compromising on the yield.

The activity of the nitrogenase enzyme complex is typically suppressed by oxygen, which is virtually present in all plant cells (Dixon *et al.,* 1997). In legume nodules it is sequestered by the leghaemoglobin protein. Possible solution to this problem in rice may be limiting *nif* genes expression to rice plastids in rice, where photosynthetically produced oxygen is not present, or diurnally regulating expression in chloroplasts such that nitrogen fixation only occurs at night (Britto and Kronzucker, 2004). Ribbe *et al.* (1997) envisaged another solution like expression of oxygen-tolerant nitrogenase found in the bacterium *Streptomyces thermoautotrophicus*. But, the process of use of such unusual oxygen tolerant nitrogenase fixation in chloroplast (Ribbe *et al.,* 1997) has problems of dependence on superoxide stress (Roy and Srivastava, 2010).

Energy requirements for nitrogenase reaction need to come from cellular metabolic cycles in the form of adenosine triphosphate or ATP (Subha Rao, 1982) and for reducing every N_2 molecule 36 molecules of ATP are required (Shantharam and Mattoo,1997). Hence, localization of introduced *nif* genes within plastids / chloroplasts may have additional advantage, as the substantial energetic cost of nitrogen fixation be met directly through photosynthates (Merrick and Dixon,1984).

Considering above, it may be inferred that, the engineering of BNF in rice, though appears to be surmountable, remains a distant possibility till date. On the other hand, by contrast, simultaneous expression of a group of related enzymes that influence both nitrogen and carbon metabolisms appears to be more promising (Britto and Kronzucker, 2004). This optimism is partly because of recent successes that have been achieved in rice and other plant species through the overexpression of glutamine synthetase (GS), which catalyzes the incorporation of NH_4^+ into amino acids (Gallardo *et al.,* 1999; Habash *et al.*,2001) and glutamate dehydrognase (GDH), another enzyme that brings ammonium nitrogen into the amino acid pool (Ameziane *et al.,* 2000). Further, the enhancement of specific links between nitrogen metabolism and photosynthetic functions (Britto and Kronzucker, s2001, 2004), provides an intriguing and potential beneficial opportunity.

CONCLUSION

The natural endophytic association of rice with rhizobia (Ladha *et al.,* 1989, 1996; Yanni *et al.*,1997; Engelhard *et al.*,2000) and better growth of rice plant on inoculation of rhizobia (Yanni *et al.*,1997; Chaintreuil *et al.,* 2000; Roy and Srivastava, 2010;Yanni and Dazzo, 2010; Roy, 2013) represent major step forward in achieving the technically challenging goal of reducing dependence on the need for fertilizer-N without requiring highly developed system as the root nodule *Rhizobium*-legume symbiosis. However, this understanding only is beginning, which needed to be explored (Yanni *et al.,* 1997). The idea of nodulation in rice is also considered not as an entirely absurd notion, given the comparatively recent discoveries of rhizobial nodulation of the non-legume *Parasponia* and nonrhizobial nodulation by *Frankia* (Bennet and Ladha, 1992). Inspite of great progress has been made toward characterization of the genes involved in this process and the functions of the protein they express (Stougaard, 2001; Geurts and Bisseling, 2002; Trevaskis *et al.,* 2002), there is still large gaps in our knowledge and extensive hurdles (de Bruijn *et al.*,1995) and therefore, number of researchers opined that, more knowledge about the plant genes involved in nodulation and the symbiotic nitrogen fixation and the function of their gene products is needed before the development of true nodulation of cereals (de Bruijn *et al.*,1995; Kennedy *et al.*,1997; Reddy *et al.*,1997; Webster *et al.,* 1997). In comparison, possibly larger challenge is directly engineering nitrogen fixation in rice (Britto and Knonzucker, 2004). de Bruijn *et al.* (1995) and Dixon *et al.* (1997) too considered it even more complex. According to Stoltzfus *et al.*(1997), though most of the genes necessary for nitrogen fixation in bacteria are well characterized, the transfer of the genes to the plant genome, along with the appropriate expression of all these genes is beyond the current technical ability. In addition, the creation of the proper environment, in terms of oxygen concentration / supply, energy provision to the bacteria, and efficient ammonia assimilation within the plant cells present serious problems (de Bruijn, *et al.,* 1995; Dixon *et al.,* 1997). Therefore, it will likely require many years of intensive research and development before a useful product making for field trial is possible (Britto and Kronzucker, 2004). On the other hand, among the three choices, it appears that, employment of endophytic nitrogen fixing bacteria involve fewest technical challenges (Stoltzfus *et al.,* 1997). Hence, in the near future, enhancing rice nitrogen status by optimizing associations between rice and naturally colonizing endophytic bacteria may be more promising (Britto

and Kronzucker, 2004). Even if an identified stably endophytic microbe do not have the capacity to fix nitrogen, the process of introducing and expressing the *nif* gene complement in such an endophyte would be significantly easier than engineering the rice plant itself to fix nitrogen (Stoltzfus *et al.*, 1997).

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RESEARCH ARTICLE

EFFECT OF DIFFERENT THERMAL ENVIRONMENTS ON THE GROWTH AND DEVELOPMENT OF WHEAT VARIETIES FOR CHHATTISGARH PLAIN

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Abstract: The effect of different thermal environments on the growth and development of wheat varieties for Chhattisgarh Plain. Higher number of ear heads/m^2 observed with 05 December and 15 December sowing may be due to favorable temperature conditions during tillering stage i.e., maximum was below 28°C, minimum was below 12°C and mean temperature was below 20°C. Length of ear heads of different wheat varieties was influenced due to temperature and shifting thermal environment. Longer ear head (9.4 cm) was observed in first and second date of sowing (25 November and 05 December) as compared to delayed sowing of 15 December, 25 December and 05 January. Longer ear head was observed in variety GW-273 (9.4) while minimum (8.4) was observed in Amar. The maximum number of grains/ ear head was observed in D_1 (52) as compared to other sowing dates (D_2, D_3, D_4 and D_5). The test weight of different wheat varieties was influenced significant by different thermal environment and delayed sowing ultimately resulted in lower test weight. On an average the higher test weight (40.1) was observed in D_1 (25 November) at par with 05 December sowing as compared to late sown condition. Maximum grain yield 3307 kg/ha was harvested in 2nd (05 December) date of sowing which was significantly higher as compared to before and delayed sowing. On an average significant higher grain yield was obtained in variety Kanchan (3190 kg/ha) followed by GW-273 whereas, the lower grain yield was recorded in variety Amar (2609 kg/ha) and Sujata (2740 kg/ha) being at par to each other. Higher straw yield (4667 kg/ha) was observed in Kanchan under 2nd sowing date (05 December) whereas the lowest (2703 kg/ha) was observed in variety Amar under 05 January sowing. On an average, in different sowing dates significantly, highest straw yield (3964 kg/ha) was recorded in variety Kanchan as compared to other varieties.

Keyword: Grain yield, Straw yield, Thermal environments

INTRODUCTION

Wheat (*Triticum spp*.) is the major Rabi crop in India and is sensitive to various biotic and abiotic stresses like weather and inter-seasonal climatic variability (in terms of changes in temperature, rainfall, radiation), soil conditions and agricultural inputs like nitrogen, water and pesticides. Three main species commonly grown in the world including India are the common wheat (*Triticum aestivum)* the marconi or durum wheat (*Triticum durum*) and the emmer wheat (*Triticum dicoccum*), out of these species maximum area is under *Triticum aestivum*. In India, more than 80 percent of the total wheat area is under this species whereas the area under marconi and emmer wheat, the area is only 12 per cent and 1 per cent respectively.

Bishnoi (2002) a field experiment was conducted during 1998-99 winter season with eight wheat varieties and three dates of sowing in split plot design with three replications. The magnitude of linear growth rate and duration of its phase largely determined the dry matter accumulation. Duration of linear phase was cultivar specific and strongly influenced by radiation, sunshine hours and saturation deficit, and explained about 70 per cent of its variations. Also sowing to anthesis and anthesis to maturity duration's were explained to 75 and 93 per cent variations when solar radiation, sun shine hours, saturation deficit were taken in to account.

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Aslam *et al*. (2003) reported that less number of tillers in late sowing was the result of less germination count per unit area which occurs due to low temperature. In case of delayed sowing the temperature was not according to the tillering requirement which results in less number of tillers m^{-2}. Differences in number of tillers m^{-2} among varieties might be attributed to their genetic diversity.

MATERIAL AND METHOD

Experimental Detail

The experiment consisting of 5 date of sowing and 4 wheat varieties were laid out in a randomized block design with three replications.

Post harvest observations

Number of ear heads per m^2

Number of ear heads were counted before harvesting from 3 randomly plots of 1 m^2 earlier fixed to record plant population, then averaged.

Length of ear head

Length of 10 ear heads randomly selected in each plot was measured from base to tip of ear head excluding awns and then averaged.

No. of grains per ear head

The ear heads used to measure the length were threshed and number of grains were counted and averaged.

Grain and Straw yield

The crop from each net plot was harvested and tied. The total biomass weight (grain and straw) was obtained by weighing the bundles after proper sun drying. The grain from each bundle was threshed out and the weights of cleaned grains were noted. The straw yield was obtained by subtracting the grain yield from the total biomass of respective treatments.

Statistical analysis

All the data were tabulated and analysed statistically as per the procedure suggested by Panse and Sukhatme (1967) and Chandel (1984). The F test was used for judging the significance of the treatment mean at 5% level. Whenever F test showed significant difference the differences between treatment means were further tested by using critical difference (CD) value.

To compare different mean value of treatments, critical difference (CD) values were calculated as follows:

$$\mathbf{SEm \pm} = \sqrt{\frac{\mathbf{Ems}}{\mathbf{n}}} \quad \text{(i)}$$

Where,

SEm ± = Standard error of mean

Ems = Error mean square

n = Number of observations on which the mean values is based

(ii) CD (5%) = SEm x$\sqrt{2}$ x t at 5 % for Error d.f. (2.02).

Regression equation were also worked out to find out the differet weather parameters on grain yield.

RESULT AND DISCUSSION

Growth parameters

Plant population

The data summarized the plant population per unit area under different thermal environments and varieties are shown in Table 1. It can be seen from the table that mean plant population was significantly higher with variety Sujata which was at par with Kanchan and Amar. Lowest plant population (212) was observed with variety GW-273, which may be due to higher seed index of this variety. The data (Table 1) showing plant population per m^2 area under different dates of sowing revealed that plant population influenced significantly due to different thermal environments. Significantly higher plant population was recorded with the crop sown on 05 and 15 December because of favorable temperatures during this period. The mean temperature during 05 and 15 December was near 20°C whereas during earlier or late sowing it was below 20°C. Aslam *et al*. (2003) reported that less number of tillers in late sowing was the result of less germination count per unit area which occurs due to low temperature. In case of delayed sowing the temperature was not according to the tillering requirement which results in less number of tillers m^{-2}.

Plant height

The plant height is the best measures and index of the total performance and response of the crop to the weather condition. Response of wheat varieties on different thermal environment are given in table 2. The plant height of the different varieties at 10 days intervals from 30 DAS to maturity are sown in Table 2 and also depicted though fig. 1 and 2. It can be seen that the initial plant height up to 30 DAS was higher in GW-273 (37.4 cm) followed by Kanchan (35.6 cm). It was observed that the plant height increased rapidly from 40 DAS to 60 DAS that is till the crop entered reproductive stage. There after the increase in height was marginal. At maturity Sujata attained maximum height of 105.0 cm followed by Amar (101.0 cm) when the crop was sown on 05 December. GW-273 attained the highest height of (85.8cm) under 05 December followed by Kanchan (84.7 cm) in case of 25 November sowing. Regarding the thermal effect on plant height Sujata expressed the highest effect as the height reduced from 105.0 cm to 91.2 cm under D_2 to D_5, the same trend was followed by Amar (101.0 cm to 92.3 cm). GW-273 variety was least affected due to thermal stress where the decrease in height was from 85.8 cm to 80.5 cm i.e., 5.3 cm only.

It was observed that the plant height increased with advancement in crop growth and reached to maximum at maturity. The increase in plant height took place slowly up to 40 days after sowing. Maximum rate of increase in plant height was observed between 40 to 60 days after sowing when the crop was in ear emergence stage. In general, higher plant height was observed in varieties Sujata and Amar and lower plant height was recorded in varieties Kanchan and GW-273. It was observed that plant height decreased from the first date of sowing when the sowing was delayed of 25 November to 05 January. It was also observed that the average plant height varied among the varieties because of the genotypic characters of these varieties (Table 2, Fig. 1 and 2). Shahzad *et al*., (2002) found that the decrease in plant height in late sowing was due to shorter growing period. Early sown crop may have enjoyed the better environmental conditions especially the temperature and solar radiation which resulted to tallest plants. Naik *et al.*, (1991) and Behra (1994) also reported decrease on plant height under delayed sown conditions.

Dry Matter Production

The accumulated dry matter at 10 days interval from sowing to maturity for all the four varieties in respect of different thermal environments are shown in Table

3 and fig. 3 and 4. The data presented in table 3 revealed that at initial stage the dry matter was higher under 05 December sowing. The dry matter production was variable at 40, 50 and 60 days after sowing as well as later dates. Further it was observed that the rate of increase in dry matter was slow up to 40 days after sowing and continued up to maturity in all the varieties under all the sowing dates except last sowing date i.e. 05 January where it was only up to 80 days after sowing. The highest dry matter was observed at maturity with Kanchan (809.8 g/m^2) under 25 November sowing while lowest dry matter was observed in variety Amar (476.5 g/m^2) under 05 January sowing. The dry matter growth rate varied differently for different varieties. The dry matter decreased sharply at maturity when sowing was delayed beyond 15 December as the maximum, minimum and mean temperature increased considerably and recorded above 30°C under 25 December and 05 January sowing. It was also reported by may part workers that mean temperature above 30°C was not favorable for optimum growth of wheat crop during maturity stage. Decreases in dry matter due to thermal stress from D_2 to D_5 as compared to D_1 can be seen from the Table 3. The dry matter production decreased considerably in all the varieties, when sowing was delayed from 25 November to 05 December, 15 December, 25 December and 05 January. However, different varieties exhibited different trends of decrease in dry matter. At maturity lowest decrease in dry matter was observed in Sujata (137.2 g/m^2) in D_5 and it was maximum in GW-273 (246.7 g/m^2) in D_5. The biomass production is mainly governed by genetic potential of the varieties under given set of weather conditions. But, the extent of decline in dry matter under delayed sowing was due to the thermal stress tolerance capacity of a particular variety to higher temperatures. So, decrease in dry matter of Sujata was found to be more tolerant to thermal stress. Low temperature under late seeding delayed germination and hampered tillering which produced less number of leaves and consequently led to poor dry matter production than timely sown crop (Pal *et al.*, 1996). Rahman *et al*., (1997) also reported similar results.

CONCLUSION

Plant population per m^2 area under different dates of sowing revealed that plant population influenced significantly due to different thermal environments. Significantly higher plant population was recorded with the crop sown on 05 and 15 December because of favorable temperatures during this period. The mean temperature during 05 and 15 December was near 20°C whereas during earlier or late sowing it was below 20°C. It was observed that the plant height increased with advancement in crop growth and reached to maximum at maturity. The increase in plant height took place slowly up to 40 days after sowing. Maximum rate of increase in plant height was observed between 40 to 60 days after sowing when the crop was in ear emergence stage. In general, higher plant height was observed in varieties Sujata and Amar and lower plant height was recorded in varieties Kanchan and GW-273. The highest dry matter was observed at maturity for Kanchan (809.8 g/m^2) while lowest dry matter was observed in varieties Amar (476.5 g/m^2). The dry matter growth rate varied differently for different varieties. The dry matter decreased sharply at maturity when sowing was delayed beyond 15 December as the maximum, minimum and mean temperature increased considerably.

Higher number of ear head /m^2 (353) was observed 05 December sowing as compared to 25 November, 25 December and 05 January sowing (339, 305 and 234, respectively) but significantly at par with that with 15 December sowing (350). Ear heads/m^2 decreased slowly up to 25 December and after it decreased sharply when sowing was done on 05 January (D_5). Length of ear of different wheat varieties was influenced due to temperature and shifting thermal environment. Longer ear head (9.4 cm) was observed in first and second date of sowing (25 November and 05 December) as compared to delayed sowing of 15 December, 25 December and 05 January. Longer ear head was observed in variety GW-273 (9.4) while minimum (8.4) was observed in Amar. Number of grain/ear head with 05 December sowing was found next that of 25 November sowing but significant superior over 15 and 25 December sowing. Significantly lower number of grains/ear head was observed with 05 January sowing. Among different variety GW-273 produced significantly higher number of grains/ear head closely followed by Kanchan being at par to each other (50 and 48, respectively) Sujata and Amar produced lower number of grains/ear head and were found statistically similar to each other.

Maximum grain yield 3307 kg/ha was harvested in 2nd (05 December) date of sowing which was significantly higher as compared to before and delayed sowing. On an average significant higher grain yield was obtained in variety Kanchan (3190 kg/ha) followed by GW-273 whereas, the lower grain yield was recorded in variety Amar (2609 kg/ha) and Sujata (2740 kg/ha) being at par to each other. This may be attributed to the fact that sowing of wheat on 05 December and 15 December provided sufficient period for vegetative growth of the crop and favorable temperature resulting in higher yield. The test weight of different wheat varieties was influenced significant by different thermal environment and delayed sowing ultimately resulted in lower test weight. On an average the higher test weight (40.1) was observed in D_1 (25 November) at par with 05 December sowing as compared to late sown conditions.

Higher straw yield (4667 kg/ha) was observed in Kanchan under 2nd sowing date (05 December)

whereas the lowest (2703 kg/ha) was observed in variety Amar under 05 January sowing. On an average, in different sowing dates significantly, highest straw yield (3964 kg/ha) was recorded in variety Kanchan as compared to other varieties. Straw yield influenced by sowing dates and temperature.

Among the four varieties, GW-273 was found to be moderately susceptible while other varieties are susceptible for thermal stress; this might be probable reason for reduction total duration and stunted crop growth. Based on the above results it was concluded that under Raipur conditions, if the sowing was delayed after 15th December GW-273 varieties should be sown to get higher yield.

Table 1. Plant population of wheat varieties as influenced by different thermal environments

Varieties	Plant population/m^2					
	D_1	D_2	D_3	D_4	D_5	Mean
Kanchan	216	226	246	211	210	222
GW-273	210	248	215	195	191	212
Sujata	200	250	238	225	215	226
Amar	215	243	240	211	203	222
Mean	210	242	235	211	205	
	SEm+/-	**CD (P=0.05)**	**CV (%)**			
D	3.5	9.9	5.4			
V	3.1	8.9				
DXV	7.0	19.9				

Table 2. Plant height (c.m.) per plant of wheat varieties at 10 days intervals under different thermal environments

Sowing dates	Days after sowing							
	30	40	50	60	70	80	90	Maturity
Kanchan								
D_1-25 Nov.	35.6	47.2	64.6	77.7	79.5	82.1	83.0	84.7
D_2-05 Dec.	34.3	41.6	55.8	71.3	73.3	78.0	78.2	80.3
D_3-15 Dec.	34.0	39.4	52.1	66.2	73.4	77.7	79.6	80.0
D_4-25 Dec.	31.6	40.8	50.6	69.1	69.8	75.0	75.4	77.1
D_5-05 Jan.	24.8	38.7	46.0	57.0	67.6	68.0	69.6	74.1
GW-273								
D_1-25 Nov.	37.4	44.2	61.3	74.3	76.7	78.0	82.8	84.6
D_2-05 Dec.	31.4	49.8	64.1	75.7	78.2	80.6	83.3	85.8
D_3-15 Dec.	31.0	40.4	53.2	68.7	72.4	76.7	80.2	82.6
D_4-25 Dec.	30.3	38.4	51.5	68.0	70.9	75.1	78.7	81.9
D_5-05 Jan.	28.2	37.1	46.4	58.7	67.2	72.4	78.5	80.5
Sujata								
D_1-25 Nov.	30.4	52.8	60.1	78.0	90.7	94.0	95.5	98.7
D_2-05 Dec.	37.2	54.2	66.7	81.3	93.4	101.4	103.0	105.0
D_3-15 Dec.	30.4	42.0	56.7	75.8	80.6	91.6	94.5	96.4
D_4-25 Dec.	30.0	40.3	48.9	55.8	75.5	88.7	90.4	93.5
D_5-05 Jan.	28.7	35.8	37.8	51.0	66.1	86.9	88.4	91.2
Amar								
D_1-25 Nov.	34.6	44.4	62.7	76.5	87.9	94.4	96.6	98.1
D_2-05 Dec.	31.9	51.6	61.9	76.7	94.3	97.4	99.1	101.0
D_3-15 Dec.	31.6	41.2	52.9	68.2	81.5	91.9	94.3	96.3
D_4-25 Dec.	30.0	40.5	51.4	59.7	78.4	88.4	92.0	94.4
D_5-05 Jan.	28.3	33.9	37.4	51.6	63.8	86.3	89.7	92.3

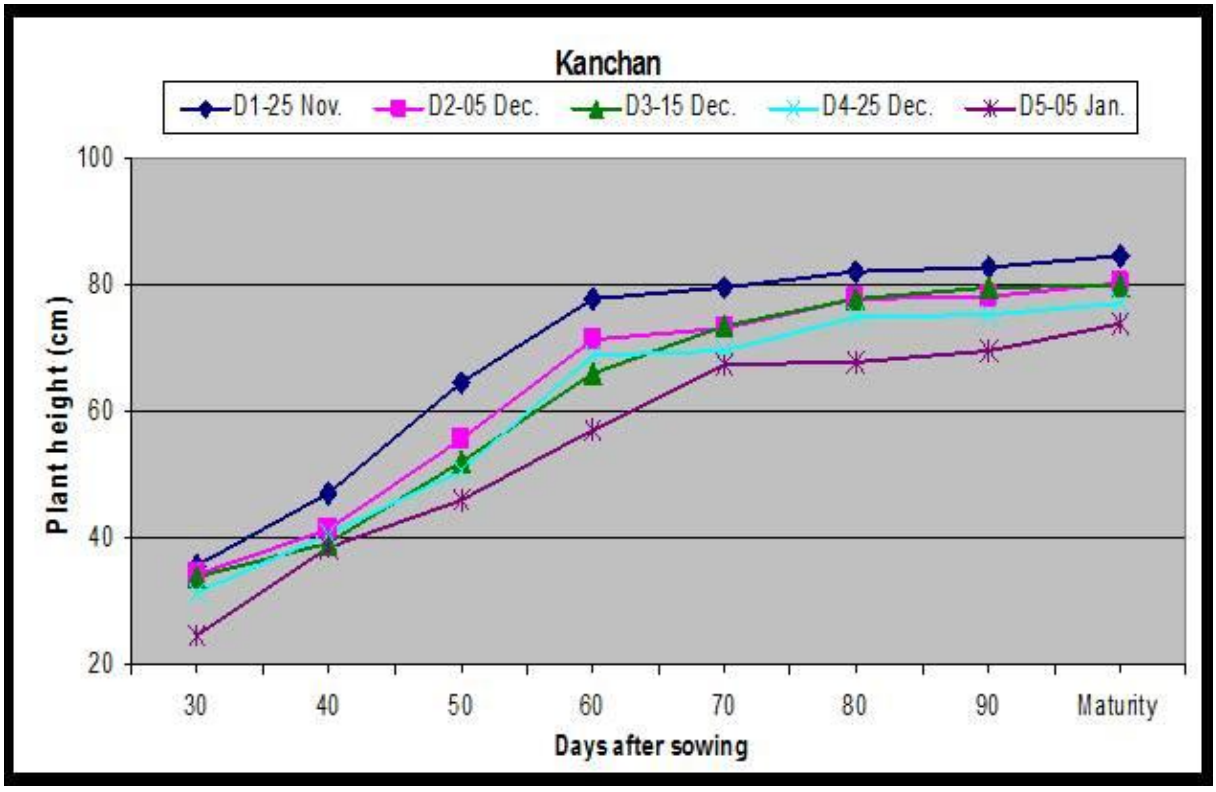
Table 3. Dry matter production per g/m^2 of wheat varieties at 10 days interval under different thermal environments

Sowing Dates	Days after sowing							
	30	40	50	60	70	80	90	Maturity
Kanchan								
D_1-25 Nov.	37.2	93.6	110.4	221.8	292.6	439.0	585.2	736.2
D_2-05 Dec.	52.0	60.1	194.2	266.9	390.1	588.9	653.7	809.8
D_3-15 Dec.	23.1	57.5	155.7	335.7	399.2	551.9	598.6	653.2
D_4-25 Dec.	34.8	78.5	156.3	431.4	453.9	496.2	576.7	621.5
D_5-05 Jan.	23.3	82.4	235.0	287.0	332.8	499.3	513.9	532.4

GW-273								
D_1-25 Nov.	45.4	100.7	112.6	290.6	322.2	376.2	631.5	773.6
D_2-05 Dec.	58.3	91.2	212.3	259.9	465.1	575.9	618.3	716.8
D_3-15 Dec.	30.1	82.9	142.8	312.4	528.7	580.0	662.0	736.8
D_4-25 Dec.	30.3	75.1	183.5	326.8	425.4	492.5	575.0	683.5
D_5-05 Jan.	26.7	99.6	206.8	259.7	326.3	476.6	484.6	526.9
Sujata								
D_1-25 Nov.	41.1	100.1	115.6	220.1	257.4	391.5	488.8	625.1
D_2-05 Dec.	41.8	79.8	176.3	208.0	323.5	468.8	561.1	666.5
D_3-15 Dec.	31.4	72.1	170.6	283.9	446.9	462.8	511.1	559.7
D_4-25 Dec.	41.5	103.1	187.0	327.9	435.7	456.6	495.3	559.9
D_5-05 Jan.	31.2	105.9	204.4	255.0	314.9	410.7	442.9	487.9
Amar								
D_1-25 Nov.	40.3	82.0	108.2	267.7	314.5	398.4	523.1	659.8
D_2-05 Dec.	50.5	82.9	137.9	247.5	336.2	438.9	577.7	626.0
D_3-15 Dec.	27.2	79.4	183.5	297.7	408.7	489.1	536.9	625.5
D_4-25 Dec.	40.0	71.5	169.6	361.9	383.1	416.2	503.9	617.2
D_5-05 Jan.	35.0	61.3	191.0	228.5	300.6	382.1	426.9	476.5

Table 4. Changes in dry matter production due to thermal stress in D_2, D_3, D_4 and D_5 as compared to D_1

S. No.	Variety	Increase/Decrease in Dry matter (g / m^2) From D_1 to			
		D_2	D_3	D_4	D_5
1.	Kanchan	+73.6	-83	-115	-203.8
2.	GW-273	-56.8	-36.8	-90.1	-246.7
3.	Sujata	+41.4	-65.4	-65.2	-137.2
4.	Amar	-33.8	-34.3	-42.6	-183.3



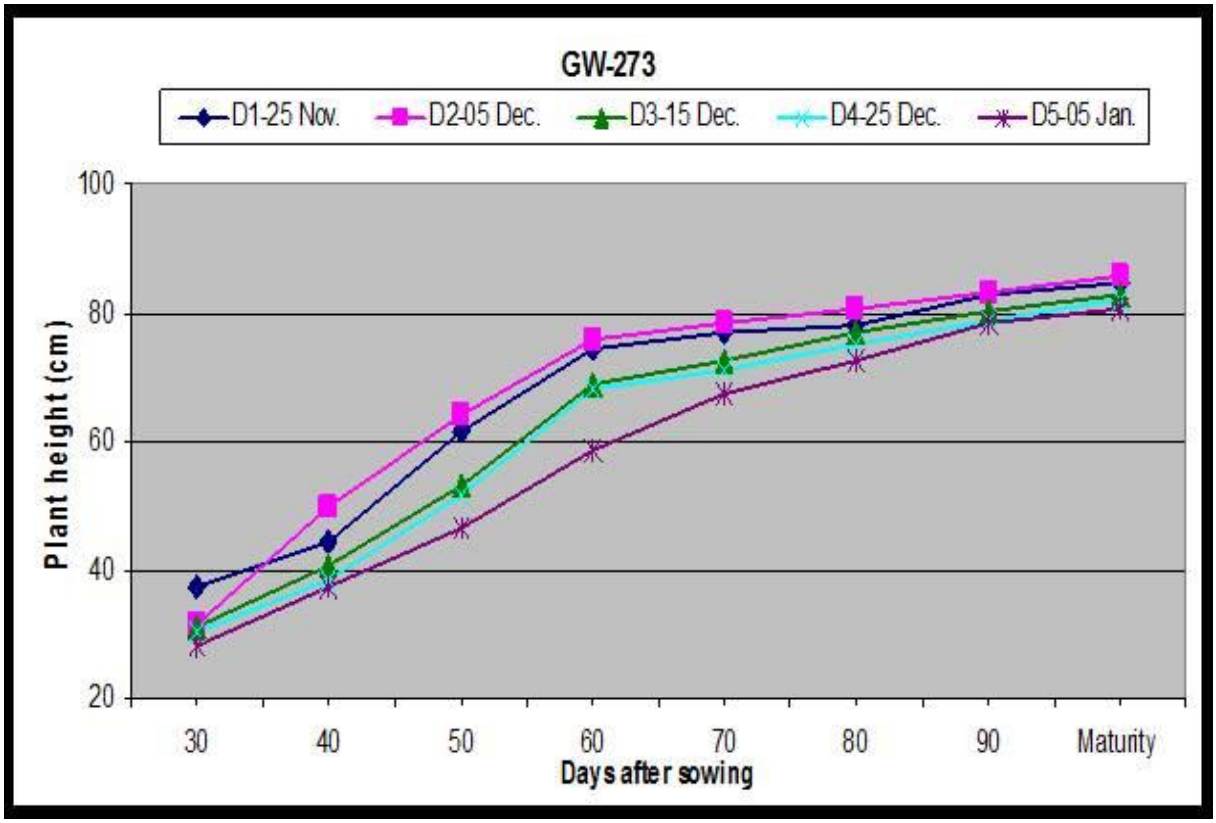
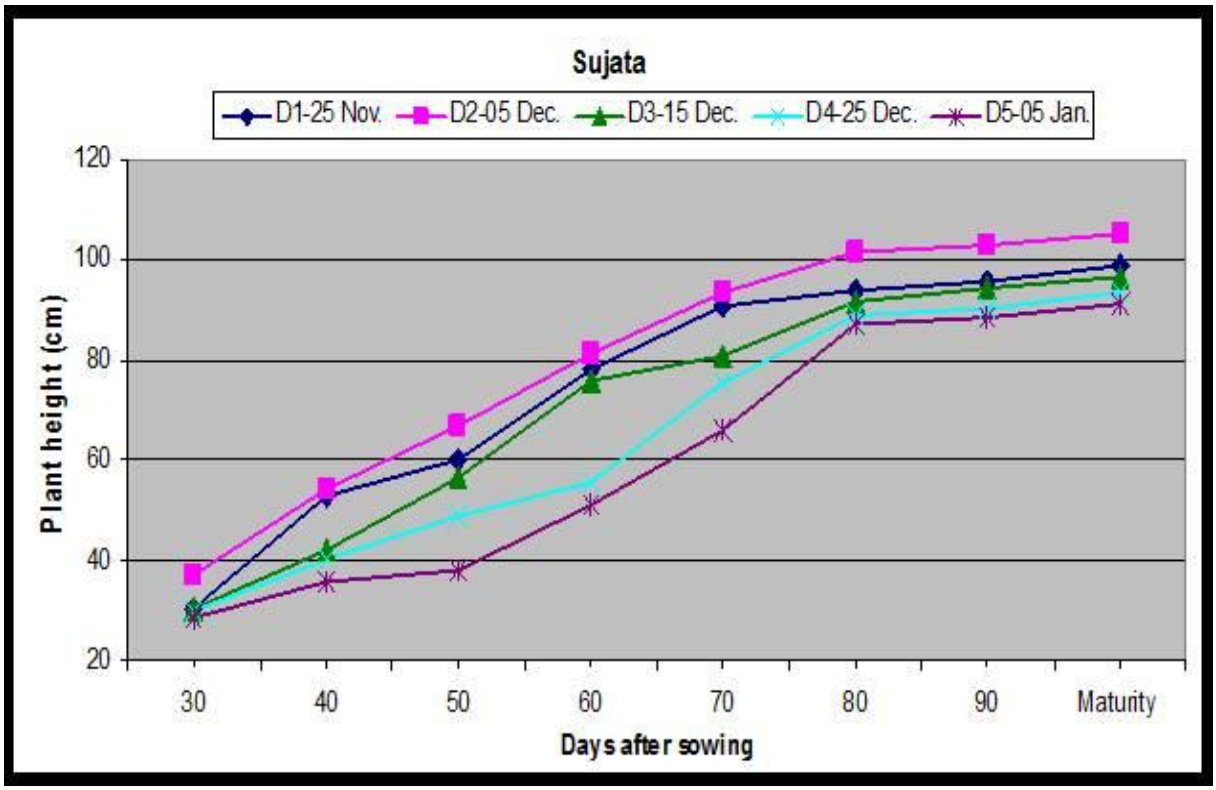


Fig.1. Plant height of varieties Kanchan and GW-273 under different thermal environments (D1 to D5)



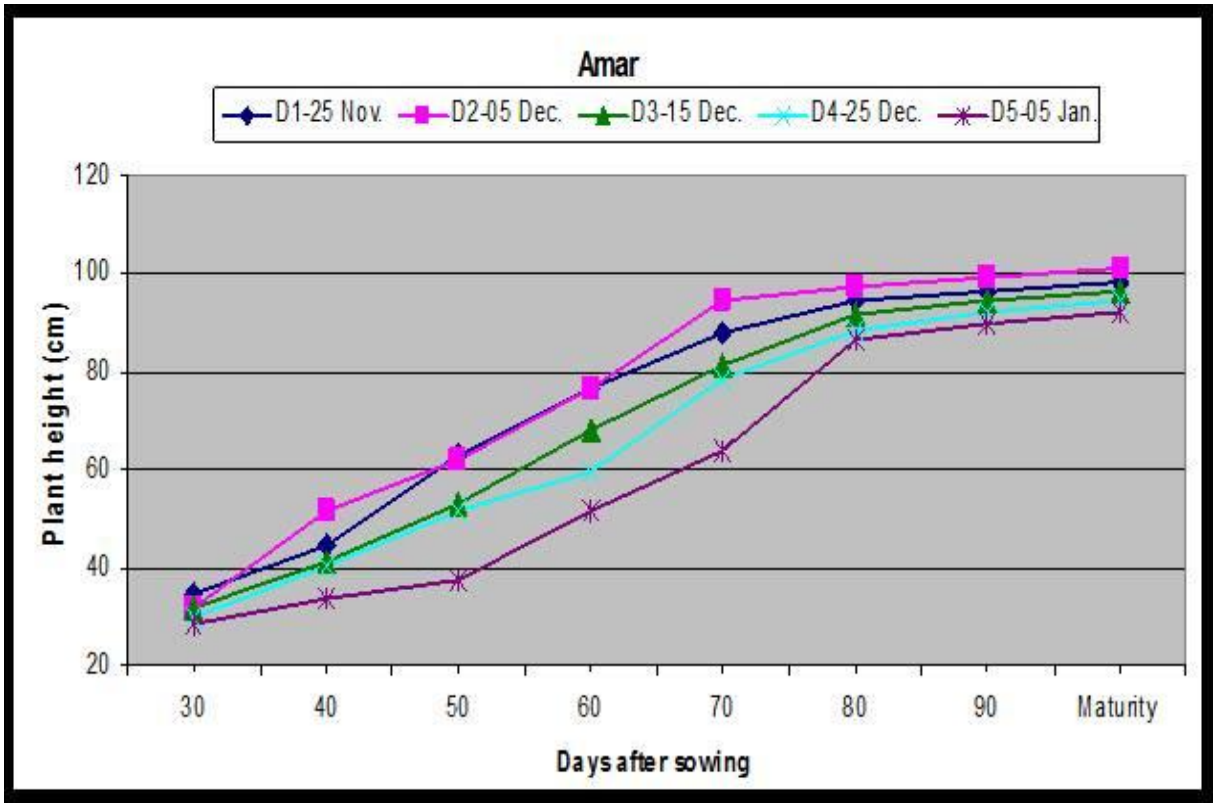
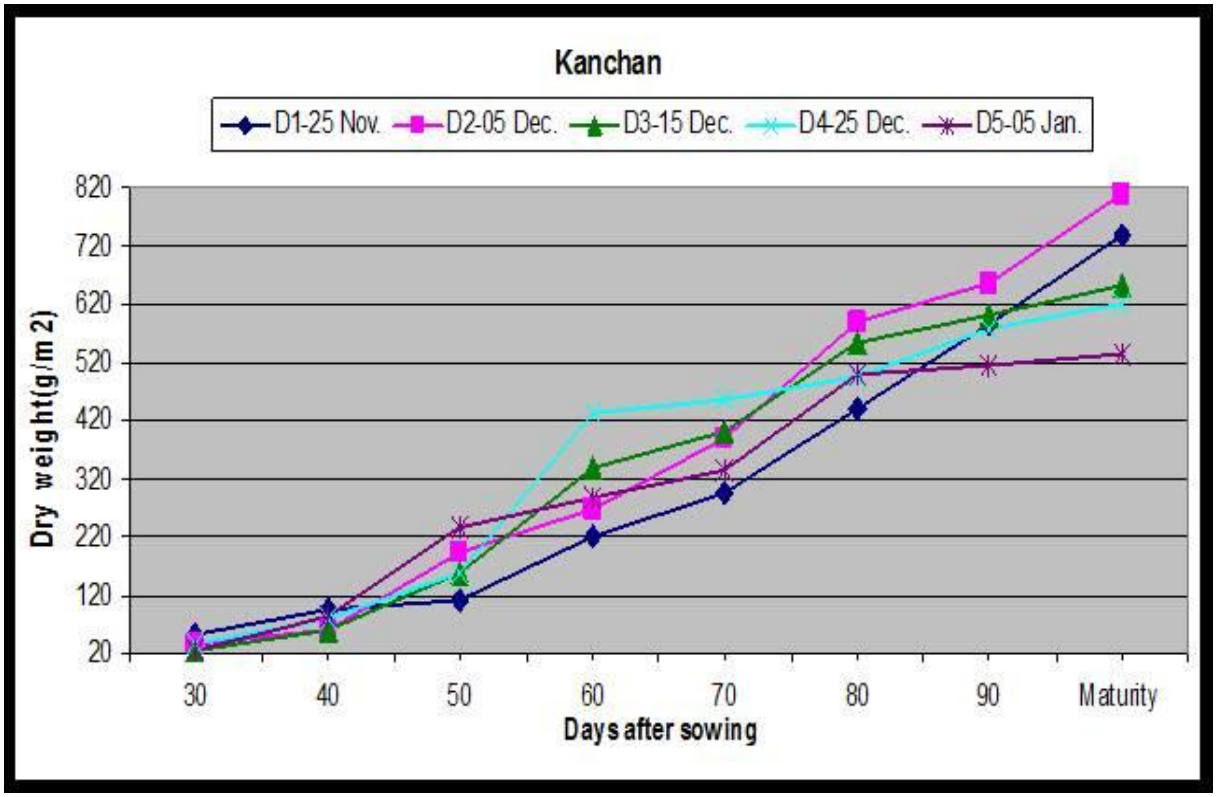


Fig. 2. Plant height of varieties Sujata and Amar under different thermal environments (D1 to D5)



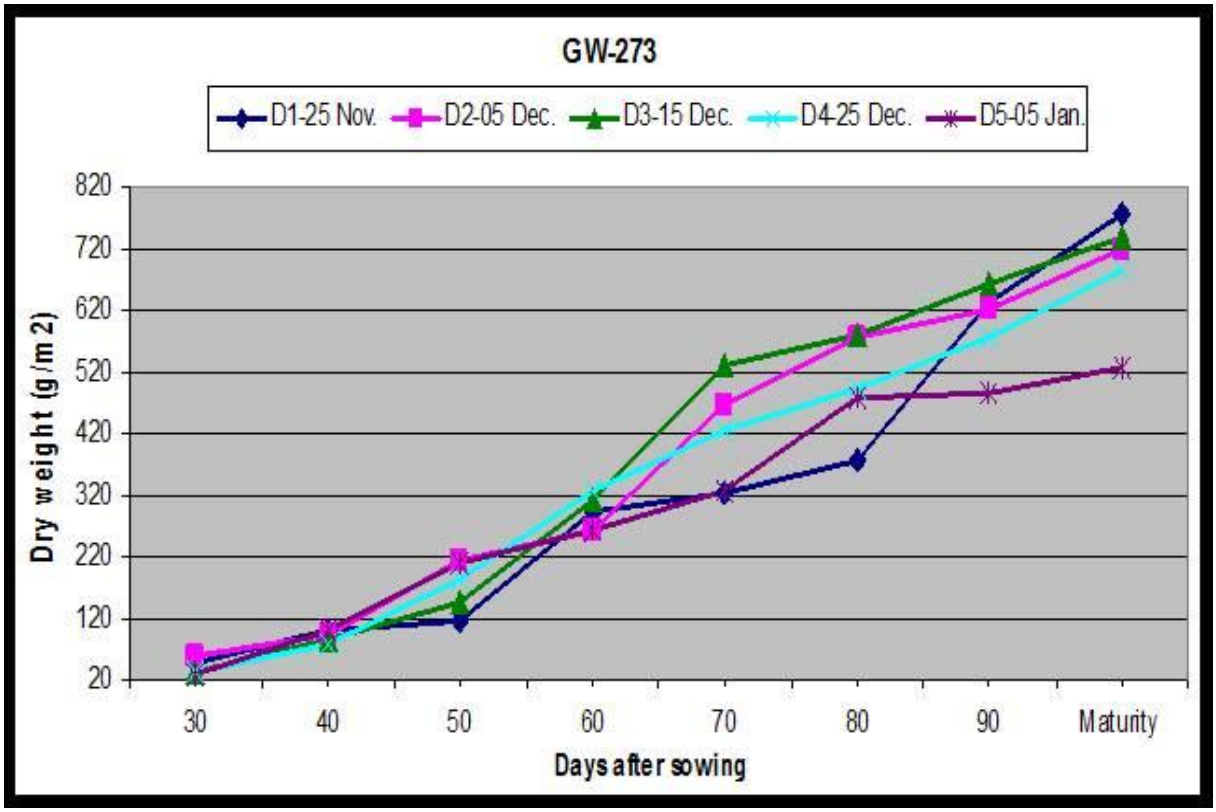
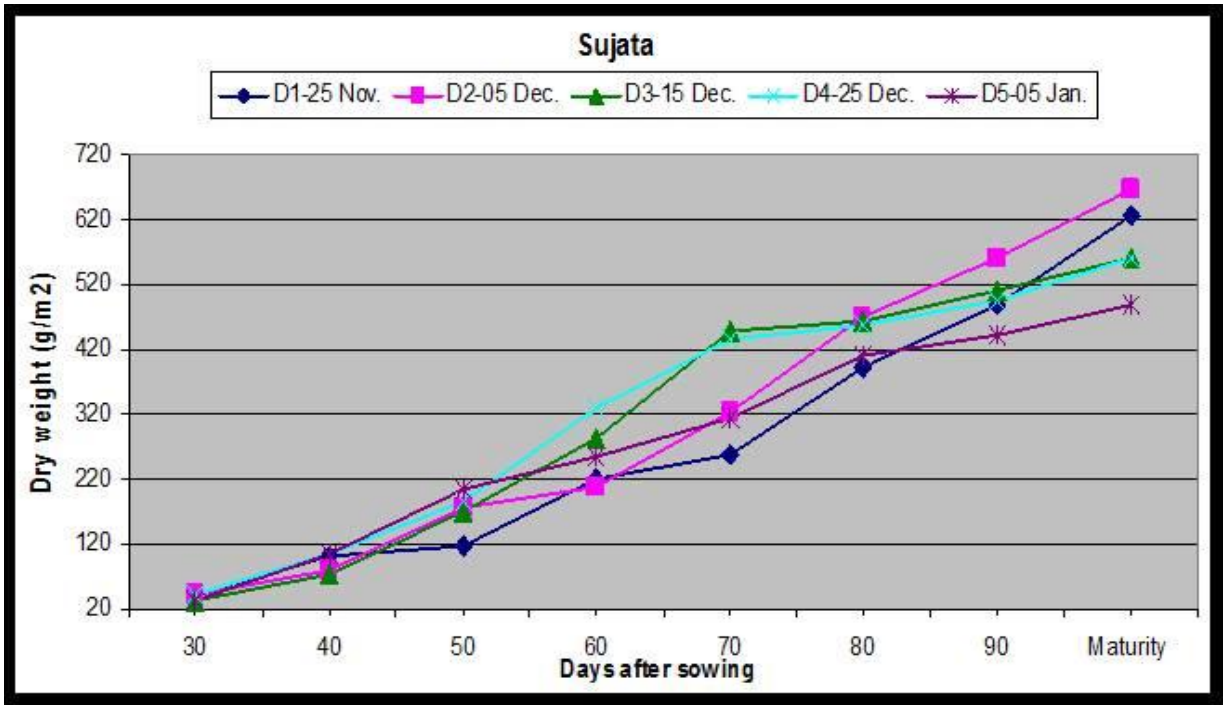


Fig. 3. Dry matter production of varieties Kanchan and GW-273 under different thermal environments (D1 to D5)



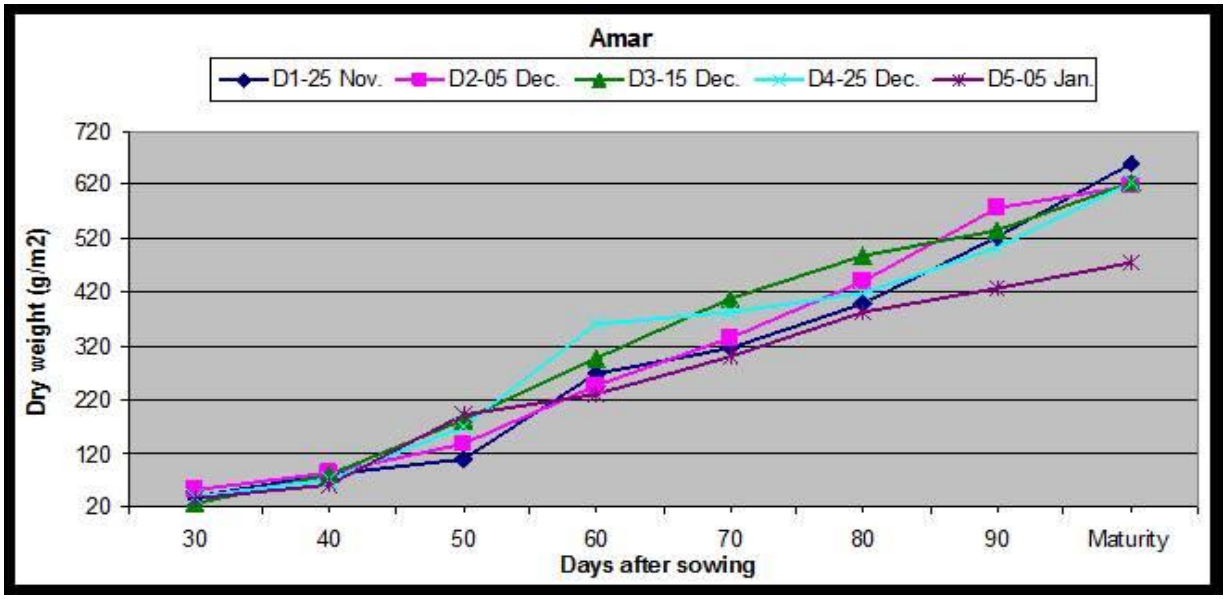


Fig. 4. Dry matter production of varieties Sujata and Amar under different thermal environments (D1 to D5)

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RESEARCH ARTICLE

COMMON INDIAN MEDICINAL PLANTS TRADITIONALLY USED FOR ANTICANCER ACTIVITY—A REVIEW

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Abstract: Cancer is an abnormal growth and proliferation of cells. It is the world second biggest killer after cardiovascular disease. Medicinal plants have been used for healing and preventative health for thousands of years all around the world. The use of herbal medicines in cancer prevention and treatment is increasing worldwide, now days because of their natural origin and lesser side effects. Traditional medicines are widely used in India. There are 21,000 plants which are used for medicinal purposes around the world as listed by World Health Organization. Among these, 2500 species are in India, out of which 150 species are used commercially on a large scale. India is called as botanical garden of the world and is the largest producer of medicinal herbs (Seth *et al*., 2004) Research indicates several possible mechanisms of action for herbal medicines and their phytochemicals may act alone or in concert to reduce cancer risk through their anti-oxidant (Ahmed *et al*., 2013), and anti-tumorigenic properties, as well as their direct suppressive effect on carcinogen bioactivities. In this article we gather the information about the easily available plants used previously and recently identified in the treatment of cancer.

Keywords: Ayurveda, Cancer, Medicinal plants, Treatment

INTRODUCTION

Cancer is the second leading cause of death, after cardiovascular disease (WHO, 2007; Mathers & Loncar, 2006). Cancer is responsible for one in eight deaths worldwide—more than AIDS, tuberculosis, and malaria together (Sener *et al*., 2005). Globally, the number of cancer deaths is projected to increase from 7.1 million in 2002 to 11.5 million in 2030 (Mathers & Loncar, 2006). Cancer patients who already got crippled with this disease further burden by drug-induced toxic side effects. Chemotherapy is routinely used for cancer treatment. Cancer cells lose a lot of regulatory functions present in normal cells, they continue to divide but normal cells do not. Recently the discovery of active components from the plant and their biological function in disease control has led to active interest in the plants across the globe. Ayurveda therapy was found to be able to cure these chronic diseases better, which were previously not amenable to treatment by western medical practices. This traditional Ayurvedic Indian therapy with its evolution through centuries has always fascinated practitioners and researchers for its applications in cancer treatment on a scientifically proven research background. Herbal decoctions consists of many herbs each possessing tremendous potential for a cancer cure (Treadway, 1998).

Plants and products derived from them have been proved effective and safe in the treatment of various cancers. Many natural products and plant constituents have been identified as potent anticancer agents. Anticancer properties of various plants and phytochemicals are being identified by many researchers. Several plant-based anticancer agents including taxol, vinblastine, vincristine, camptothecin derivatives, topotecan, and irinotecan are in clinical use all over the world. Plants still have tremendous potential to provide newer drugs and act as reservoir of natural chemicals that may provide chemoprotective agents against cancer. Recently, Taneja and Qazi, have suggested a number of compounds from medicinal plants with potential anti-cancer activities (Murthy, 1987). This review will discuss some of the plants and their products that have recently been tested and may have potential in anticancer therapies.

Comman plants having anticancer properties

Ashwagandha

Ashwagandha (Withaniasomnifera), is a widely used medicinal herb in Ayurveda. It is considered to be a *rasayana* herb, an adaptogen (which adapt themselves to the need of the organism), and is commonly referred to as 'Indian ginseng'. The leaf and root extracts of Ashwagandha reduce the growth of breast, central nervous system, colon, and lung cancer cells without affecting normal cells. Ashwagandha also appears to make tumor cells more sensitive to radiation or heat therapies (Devi,1996; Devi & Sharada, 1996). Anticancer effect of Ashwagandha is generally attributable to steroidal lactones collectively referred to as withanolides and among them withaferin A (WA) appears most active against cancer.

In a study it was found that, withanone obtained from leaf extracts of ashwagandha, act as tumor inhibitory factor, its components kill cancer cells by at least by five different pathways, including, GM-CFS, p53, apoptosis and death receptor signaling and G2-M

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DNA damage regulation pathway (Widodo *et al.,* 2008) It has been found that naturally occurring withanone cause inactivation of the TPX2-Aurora A complex, which has an effective role during mitosis and cytokinesis and found to be up regulated in several cancer types (Grover et al., 2011), It prevents cancer proliferation by interrupting cell division and by inhibiting the development of new blood vessels. A research on animal model for lung cancer, showed that ashwagandha supported the chemotherapeutic activity of medicine paclitaxel and its antioxidant properties reduced the oxidative stress caused by tumors (Ahmed *et al.,* 2013). During study on pancreatic cancer, researcher found that Withaferin, significantly stimulates cell death in a number of pancreatic cancer cell lines. It appears to target and limit the activity of a specific protein like (Hsp90) which helps other proteins in maturing, stimulating and promoting the growth and survival of cancer cells (Yu Yanke *et al.,* 2011). The water extracts from ashwagandha leaves, containing Withaferin A, may offer a treatment option for another deadly cancer—Glioma, the most prevalent types of brain tumor (Shah *et al.,* 2009; Kataria Hardeep *et al.,* 2011). In another experiment tumor cells (Sarcoma 180) were transplanted into mice. Administration of Ashwagandha extracts to these mice at 400 mg/Kg produced complete regression of the tumor growth (Devi *et al.,* 1992). The effect of Ashwagandha have been observed on *in vitro* human cancer cells or *in vivo* on animals, showed the potential use of ashwagandha as anticancer agent.

Aloe Vera

Aloe Vera has been used in Ayurveda from ancient time. It is a popular houseplant in India and is often called the 'miracle plant' and the 'natural healer'. Aloe Vera (inner gel) contains the 8 essential amino acids that the human body needs but cannot manufacture, complex carbohydrates, enzymes, steroids, antibiotic agents and minerals. Research shows strong immunomodulatory and antitumor properties for Aloe Vera polysaccharides. It boosts immune system function while destroying cancer tumors. One study published in *International Immunopharmacology* (1995) showed that Aloe Vera polysaccharides exhibited potent macrophage-activating activities by producing increased volumes of nitric oxide (which has antitumor potential). Aloe constituents Emodin and Rhein also produced anticancer effects, which included the halting of tumour progression and cell death among cancer cells The polyphenols, glucans and alkaloids give Aloe its tremendous anti-inflammatory and antioxidant properties, along with its ability to stimulate healing.

In the books entitled "Cancer can be cured" (2000) and "Aloe in not medicine yet it cures" (2009), Father Romano Zago showed the world that curing cancer does not have to be expensive. His simple 3 ingredient formula when prepared correctly has been reported to cure many types of cancers (EJerome, 2011) It is known to reduce radiation induced damage. The majority of cancer patients who have steadily used an Aloe Vera gel throughout their radiotherapy treatment have experienced great relief as it helped to heal and sooth their skin that's why many specialist nurses and radiotherapists recommend its use, during and for a few weeks after radiotherapy treatment (Richardson *et al.,* 2005; Surjushe *et al.,* 2008). Researcher studied the effect of Aloe Vera crude extract (ACE) alone or in combination with cisplatin in human breast (MCF-7) and cervical (HeLa) cancer cells and found that Aloe Vera may be an effective anti-neoplastic agent to inhibit cancer cell growth.It increases the therapeutic efficacy of conventional drugs like cispolatin (Hussain *et al.,* 2015).

Papaya

Carica papaya is a fast growing, soft woody, herbaceous plant reaching 3-10 m in height. In some places it is known by the name pawpaw. Its leaves have been used in traditional medicines for treatment of various types of diseases like malaria, dengue fever, asthma, beriberi and cancer (Rahman *et al.,* 2011; Ahmad *et al.,* 2011). Researchers have found that papaya leaf extract and its tea have dramatic cancer-fighting properties against a broad range of tumour's including cervix, breast, liver, lung, and pancreatic (Fauziya *et al.,* 2013). Papaya Leaves have a milky sap that's great for preventing and killing cancer cells because it contains acetogenin, that supports the body's normal cells during the time of cellular stress. University of Florida, United States researcher Nam Dang and colleagues in Japan, in a report published in the Journal of Ethnopharmacology, reported the papaya's anticancer effect against tumours of the cervix, breast, liver, lung and pancreas. Dang and the other scientists showed that the leaf extracts of papaya boosts the production of key signaling molecules called Th1-type cytokines, which helps in regulating the immune system. This could lead to therapeutic treatments that use the immune system to fight cancers (Otsuki *et al.,* 2010).

Dried papaya leaf powder contain papain enzyme, which have powerful digestive action . Researchers while doing their research on action of different enzymes in treatment of cancer , found that papain will work more aggressively than the pancreatic enzymes in attacking and destroying cancer cells (Indran *et al.,* 2008). The dried leaves of papaya have been boiled for preparing tea for cancer patients. Papain enzyme was found to be effective at 150-160 degree F. The seeds of Papaya contains agents that stop the growth of cancer cells and tumors. Papaya Seeds contain isothiocyanate which works well for colon, breast, lung, leukemia and prostate cancer (Nguyen *et al*., 2013).

Giloy

Tinosporacordifolia, also known as guduchi in Sanskrit, giloya in Hindi and heartleaf moonseed plant in English, it is a climbing deciduous shrub . The most commonly used part of the shrub is the stem and its roots are also comprise important alkaloids. This shrub is commonly found in India, Myanmar, Sri Lanka and China. A variety of active components like alkaloids, diterpenoid lactones, aliphatics, glycosides, steroids like tinosporine, tinosporide, tinosporaside, cordifolide, cordifol, heptacosanol, clerodanefuranoditerpene, diterpenoidfuranolactonetinosporidine, columbin and β-sitosterol. have been isolated from the different parts of the plant body, including root, stem, and whole plant. The plant may also contain phytoandrogen, which means it can protect against DNA damage induced by the environment and radiation therapy.

T. cordifolia efficaciously kills HeLa cells *in vitro*, suggesting its potential as an anticancer agent. In a study on HeLa cells, a dose-dependent increase in HeLa cell death was observed when treated with *T. cordifolia* extract as compared to the control cells (Jagetia, 1998). Enhancement in nitric oxide (NO) production by stimulation of splenocytes and macrophages indicative of anti-tumor effects (Upadhyaya *et al.,* 2011). In another study it has been shown to block the G1 phase in EAC mice and cause apoptosis by the formation of apoptotic bodies, nuclear condensation, by activating caspase-3, increased expression of pro-apoptotic gene, *Bax*, and decreased expression of anti-apoptotic gene, *Bcl-2* (Thippeswamy *et al.,* 2007). Its extracts are capable in reduction of papilloma's, tumour yield, tumour burden, and tumor weight by increasing phase II detoxifying enzymes in skin carcinoma animal models (Chaudhary et al., 2008). In another report by Singh et al, who investigated the effect of in vivo application of alcoholic extract of T. cordifolia ,on the proliferation of cancerous cells and myeloid differentiation of bone marrow hematopoietic precursor cells on a mice bearing a transplantable T cell lymphoma of spontaneous origin called as Dalton's lymphoma (DL). Their study indicates that the T. cordifolia can influence the myeloid differentiation of bone marrow progenitor cells and the recruitment of macrophages in response to tumor growth in situ. Dichloromethane extracts of TC shows cytotoxic effects owing to lipid peroxidation and release of LDH and decline in GST (Verma *et al.,* 2011). Methanolic extract of TC that were cytotoxic to human breast cancer cells failed to induce apoptosis in Vitro cell (Rumana Ahmad *et al.,* 2015).

Tulsi

'Tulsi' is considered to be the most sacred herb of India.It is called as "TheMother medicine of nature" in Ayurveda. It improves energy levels, decreases stress and also acts as an immune-booster. Its active constituent eugenol is responsible for its anticancer potential which inhibits the multiplication, migration and invasion of cancer cells and will also induce apoptosis (programmed cell death of tumors). Moreover, holy basil has a host of cancer-fighting phytochemicals such as, apigenin, rosmarinic acid, myretenal, beta-sitosterol ,carnosic acid and luteolin, These phytochemicals in tulsi increase antioxidant activity, alter healthy gene expressions, induce cancer cell death, prevent blood vessel growth contributing to cell growth and stop metastasis, which is the spread of cancer from one organ to another. (Baliga MS *et al.,* 2013; Shimizu T *et al.,* 2013)According to a recent 2013 research published in *Nutrition and Cancer,* It was shown that flavonoid compounds present in water extracts of holy basil like, orintin and vicenin protected mice against radiation-induced tumour (Uma Devi P, 2001). According to Karthikeyan *et al.,* 1999 Holy basil (Ocimum sanctum) may have the ability to prevent the early events of carcinogenesis. Studies also indicate that holy basil can repair cells damaged by oxidation and radiation, demonstrating the potential to destroy precancerous lesions and tumours. Leaves and flowering tops are used for extracting essential oil. Oil of O. sanctum has revealed the presence of five fatty acids (stearic, palmitic, oleic, linoleic and linolenic acids). It is a good source of beta carotene, calcium and vitamin C and it also contains volatile substances including estragol, linalool, eugenol, methyl chavicol , methyl cinnamate, cineole, and some other terpenes, tannins, urolic acid etc The leaves contain an essential oil, which contains eugenol, eugenal, carvacrol, methylchavicol, limatrol and caryophylline. The oil extracted from its seeds contained fatty acids and sitosterol. The roots contain sitosterol and three triterpenes Urosolic acid and oleanlic acid possess anticancer property (Zhang *et al.,* 2004; Bhavana J. *et al.,* 2016; Prakash P & Gupta. N, 2005)

Tulsi protects against toxic chemical-induced injury by increasing the body's levels of antioxidant molecules, such as glutahione, and enhancing the activity of antioxidant enzymes. These enzymes protect cellular organelles and membranes by fighting free radical damage caused by a lack of oxygen and other toxic agents (Banerjee *et al*., 1996). Kim *et al*., 2010 found that ethanolic extract of O. sanctum can be a potent anti-metastatic candidate which inactivate matrix metalloproteinase-9 (MMP-9) and enhance antioxidant enzymes. Magesh *et al*., 2009 demonstrated thatethanolic extract of O. sanctum induces apoptosis in A549 cells via a mitochondrial caspase-dependent pathway and inhibits the in vivo growth of Lewis lung carcinoma animal model. Tae-kyungKwak *et al.,* 2014 also favour anti-metastatic mechanism of EEOS mediates inhibition of PI3K/Akt in Osteopontin (OPN) treated NCI-H460 non-small cell lung cancer cells.

Turmeric

Turmeric has been used for centuries in Ayurvedic and Chinese medicines to treat inflammation and cure infections.Turmeric contains a class of compounds known as the curcuminoids, comprised of curcumin, demethoxycurcumin and bisdemethoxycurcumin (Jurenka JS, 2009). Curcumin is the principal curcuminoid and comprises approximately 2-5% of turmeric; it is responsible for the yellow color of the spice as well as the majority of turmeric's therapeutic effects (Chattopadhyay *et al.,* 2004). Aside from being employed as a flavoring and coloring agent in food, turmeric has also been widely used in Ayurvedic medicine for its anti-oxidant, antiseptic, analgesic, antimalarial and anti-inflammatory properties (Aggarwal *et al*., 2007). Studies have shown that curcumin helps prevent several forms of cancer including oral, breast, lung, stomach, liver, and colon because of its anti-inflammatory and antioxidant properties (Coleman *et al.,* 2015; Nahar *et al.,* 2014; Elattar & Virji, 2000). Epidemiological studies attribute the low incidence of colon cancer in India due to the chemopreventive and antioxidant properties of diets rich in curcumin (Mohandas KM & Desai DC, 1999). Curcumin has been shown to suppress the expression of cyclin D1 in many types of cancer including head and neck, colon, bladder, cervical, breast, and pancreatic. And this attributes the effect of curcumin's in inhibition of NF-κB activation and subsequent suppression of downstream gene products (Liu Q *et al.,* 2009).

Cucurmin stops the development of cancer by interfering with the cellular signalling pathways. It has the ability to block cancer cells at every stage of cancer development, from cell mutation, to tumour growth, and up to metastasis. In another study it was found effective inhibitor of cancer cells by triggering apoptosis (programmed cell death) without affecting normal cells (O'Sullivan-Coyne *et al.,* 2009; Aoki H *et al.,* 2007). Ludwig Maximilians University in Munich, Germany in 2012 wshowed that curcumin can inhibit the formation of metastases in prostate and breast cancer. Both cancers spread throughout the body by releasing some chemical messengers like, pro-inflammatory cytokines CXCL1 and CXCL2, and curcumin alters the expression of these two damaging proteins.

Neem

Neem (*Azadirachta indica*) is a member of the Meliaceae family.It has been widely used in Chinese, Ayurvedic, and Unani medicines worldwide in the treatment and prevention of various diseases. Earlier finding confirmed that neem and its constituents play a vital role in the scavenging of free radical generation and prevention of disease pathogenesis (Amritpal Singh *et al.,* 2008). The active constituent of neem is azadirachtin and the others constituents like nimbin, nimbidin, nimbidol, sodium nimbinate, nimbolinin, gedunin,and quercetin are also effective against many diseases (Zong A *et al.,* 2012). The various components extracted from neem plant were used in traditional medicine for the cure of many diseases including cancer for centuries. The extracts obtained from its seeds, leaves, flowers, and fruits, have consistently act as chemopreventive agents and show antitumor effects in different types of cancer. . Neem ingredient shows impressive role in the management of cancer by regulating cell cycle and cell signaling pathways (Wu Chia-Mao *et al.,* 2009). Neem extracts modify the activity of various tumour suppressor genes (e.g., p53, pTEN), angiogenesis (VEGF), transcription factors (e.g., NF-κB), and apoptosis (e.g., bcl2, bax) (Elumalai P. *et al*., 2012; Raja Singh *et al.,* 2014) Another study was carried out to examine the effects of nimbolide neem constituent on apoptosis and insulin-like growth factor (IGF) signaling molecules on androgen-independent prostate cancer (PC-3) cells lines.The results of the study suggested that nimbolide acts as a potent anticancer agent by i inhibiting cell proliferation and inducing apoptotic pathway via PI3K/Akt pathway in PC-3 cells (Gunadharini *et al.,* 2011).

Azadirachtin and nimbolide bioactive components of neem have been studied extensively. The key anticancer effects of these components on malignant cells include inhibition of cell proliferation and induction of cell death The studies based on animal model established that neems chief constituents play pivotal role in anticancer management by modulating various molecular pathways including p53, pTEN, NF-*κ*B, PI3K/Akt, Bcl-2, and VEGF as mentioned above also. It is considered as safe medicinal plants which modulates the number of metabolic processes without any adverse effect on normal cells. Neem also plays role as anti-inflammatory via regulation of proinflammatory enzyme activities of cyclooxygenase (COX), and lipoxygenase (LOX) enzyme (Hossain *et al.,* 2013).

Sheesham

Dalbergia sissoo is known as Indian rosewood which is a deciduous forest tree. It is natively found in Indian subcontinent. It is called as Sheesham in comman language and is best known as premier timber tree. A number of phytochemicals like tannins, steroids, terpenoids, saponins, flavonoids and alkaloids including isoflavonoids, neoflavonoids, O-Prenylated flavonoids were extracted from it and found effective against different types of tumours. Glycosides, phenols, quinones, furans, oligosaccharides, trisaccharides and some other compounds have also been isolated from various parts of the D. Sissoo (Chihiro Ito, 2003; Rana V *et al*., 2009). In addition to uses in day today life D. sissoo plant consists of a large number of reputed medicinal properties (Shah *et al.,* 2010). The extract of D. sissoo leaves contains a large amount of

flavanoids. D. sissoo leaves extract can be used with poise to treat colorectal cancer alongwith other usual treatments with chemotherapeutic agents (Shaltout *et al*., 2011; Ghogare Pradip *et al*., 2014).

Palash

Palash (Buteamonosperma) from family Fabaceae popularly known as 'flame of the forest'. It has been widely used in the traditional Indian Ayurvedic medical system . Its various parts like leaves, stem bark, fruits, seeds, flowers and their extracts contain bioactive compounds such as triterpene, butein, butin, isobutrin, coreopsin, tannins, chalcones, glucosides, gallic acid, linoleic acid, palmitic and lignoceric acid were used against various disorders like diabetes, cancer and helminthic infections (Choedon T *et al*., 2010; Sehrawat *et al*., 2012; Mishra MK, 2016).

Aqueous extract of Buteamonosperma inhibit cell proliferation and arrest cells in G1 phase. This was accompanied by a marked reduction in the levels of activated Erk1/2 and SAPK/JNK which induces apoptotic cell death. In MCF-7 cancer cells, 1µM of butein is able to reduce mRNA levels of COX2 at baseline (associated with reduced ERK1/2 and PKC phosphorylation) (Lau GT *et al.*, 2010). Intraperitonial application of the aqueous extract from flowers of Butea monosperma tested on X-15-myc onco mice, this study showed antitumorgenic activity, these results suggest that the extracts may have potential chemopreventive efficacy (Rasheed Z et al., 2010). J.Banu Rekha et.al., in 2011 reported the antitumor activity of the ethanol extract of leaves of Butea monosperma (L) Taub in Ehrlich ascites carcinoma (EAC) cells bearing mice.

Hemp

The Hemp (Cannabis sativus) is an annual herb that may reach 5 meters in height and its leaves form a fan-like structure with jagged edges. It belongs to family Cannabinaceae. This plant can be used as fibre, in medicine, as a narcotic and now a day it is used to relieve cancer pain and treat depression. It is popularly known as medical marijuana. Chemical constituents of Cannabis are many hydrocarbons, sugars, terpenes, steroids ,flavonoids, nitrogenous compounds and amino acids among these cannabinoids such as tetrahydrocannabinol (THC) and cannabidiol (CBD), are mostly used in medical therapy to treat disease or alleviate symptoms, and delta-9-tetrahydrocannabinol (known as THC) is the primary psychoactive ingredient. .Indian hemp is known as *Cannabis indica*, tends to have a higher concentration of CBD than Cannabis sativa . Cannabinoids activate canabinoid receptors in human body. The human body also produces endocannabinoids which plays an important role in creating healthy environment within body cells Cannabidiol is thought to have significant pain-relieving and anti-inflammatory activity without the psychoactive effect of delta-9-THC (Tariq A.L. and A. L. Reyaz, 2012a). Cannabis oil is considered as a cancer preventer because it decreases the size of tumours and alleviate nausea, pain, lack of appetite and weakness. However, the U.S. Food and Drug Administration has not approved cannabis as a treatment for cancer but research shows that it has some anticancer properties. Both dronabinol and nabilone prepared from phytochemicals obtained from cannabis are approved for prevention and treatment of chemotherapy-induced Nausea/ Vomiting in cancer patients. In another study researchers found that cannabinoids inhibit cancer cell invasion via increasing tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) in lung cancer cell lines (Ramer R *et al.*, 2012).

Table 1. List of Plants Having Anti-Cancer Properties

S. No.	Common Name	Botanical Name	Family	Part Used	Tumor Inhibitory Factor
1	Ashwagandha	*Withania somnifera*	Solanaceae	Leaves	Withafarin-A
2	Aloe vera	*Aloe barbadensis*	Asphodelaceae	Leaves	Emodin, Cispolatin
3	Papaya	*Carica papaya*	Caricaceae	Whole Plant	Papain, Acetogening
4	Giloy	*Tinospora cardifolia*	Menispermaceae	Whole Plant	Cordifolide, Tinosporide
5	Tulsi	*Ocimum sanctum*	Lamiaceae	Leaves	Euginol, Epigenin
6	Turmeric	*Curcuma longa*	Zingiberaceae	Rhizome	Cucurmin
7	Neem	*Azadirachta indica*	Meliaceae	Bark, Leaf, Seeds	Nimbidol, Quercetin
8	Sheesham	*Dalbergia sissoo*	Fabaceae	Bark, Leaves	Terpenoids, Flavanoids
9	Palash	*Butea monosperma*	Fabaceae	Bark, Leaf, Fruits	Butein
10	Bhang	*Cannabis sativa*	Cannabaceae	Leaves	Cannabinoids

CONCLUSION

Nowadays many people are learning and doing research on ancient medicinal systems like Unani, Ayurveda and Chinese for treating various diseases. Among them the use of medicines obtained from the herbal source is found to be most effective (The Ayurveda). Ayurveda means 'the science of life'. It focuses on balancing all aspects of a human's mind, body and spirit. According to Ayurveda unbalanced Doshas (or homeostasis) affects gene expression, leading to disease like Cancer. When Dosha is in balance, Cancer doesn't occur. Due to adverse effects of chemotherapy and radiotherapy treatments and the huge cost associated with them, the use of easily available and cost effective Ayurvedic herbs that deal with all type of cancer is an effective way of treating cancer.

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RESEARCH ARTICLE

EFFECT OF TEMPERATURE ON DIFFERENT VARIETY OF WHEAT UNDER LATE SOWN CONDITION FOR THE CHHATTISGARH PLAIN

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Abstract: The least effect of thermal stress was observed in case of GW-273 (13 days). The maximum, minimum as well as mean temperature increased gradually when the sowing was delayed from 25 November to 05 January, CRI to 50% flowering and 50% flowering to maturity. At 50% flowering to maturity the maximum, minimum and mean temperature was observed as high as 40.5, 22.3 and 31.4°C for variety Amar when sown on 05 January. This showed that 34-35°C maximum, 17-18°C minimum and 26-27°C mean temperature were more favorable for higher yield of wheat crop under Raipur condition. It was observed that plant height decreased when the sowing was delayed from 25 November to 05 January. The highest dry matter was observed at maturity for Kanchan (809.8 g/m^2) while lowest dry matter was observed in varieties Amar (476.5 g/m^2). The dry matter growth rate varied differently for different varieties under different thermal environments. Temperature pattern revealed that the maximum and mean temperature was lower when the crop was sown on 25 December while the minimum temperature was lower on 05 January sowing as compared to other sowing dates from sowing to 30 days after sowing. Among the four varieties, GW-273 was found to be moderately susceptible while other varieties are susceptible for thermal stress; this might be probable reason for reduction total duration and stunted crop growth.

Keyword: Temperature effect, Thermal stress, wheat yield

INTRODUCTION

Growth and development of wheat depends up on the environment in which it is grown. Major environmental factors which influence growth and development of wheat are temperature, moisture, radiation and photoperiod. The effect of these factors is being discussed briefly according to different phenological stages of wheat. Pre anthesis period is strongly influenced in terms of spikelet and floral or kernel numbers by the environment. Sowing time is another factor, where in crop growth is decided by the environment. Even under optimum conditions small variation in temperature influenced the growth and development of wheat (Savin and Nicolas, 1996). Wheat (*Triticum aestivum* L.) is the most widely cultivated food grain crop of the world. It is grown not only in temperate zones but also in tropical and sub tropical zones. In India, wheat is the second important staple food crop, rice being the first. It has wide adaptability, and can tolerate severe cold. The best wheat are produced with cool and moist weather during the major portion of the growing period followed by dry warm weather during grain filling and maturity period. Wheat (*Triticum spp*.) is the major Rabi crop in India and is sensitive to various biotic and abiotic stresses like weather and inter-seasonal climatic variability (in terms of changes in temperature, rainfall, radiation), soil conditions and agricultural inputs like nitrogen, water and pesticides. Three main species commonly grown in the world including India are the common wheat (*Triticum aestivum)* the marconi or durum wheat (*Triticum durum*) and the emmer wheat (*Triticum dicoccum*), out of these species maximum area is under *Triticum aestivum*. In India, more than 80 percent of the total wheat area is under this species whereas the area under marconi and emmer wheat, the area is only 12 per cent and 1 per cent respectively.

Ortiz-Monasterio *et al.* (1994) found that delayed emergence of seedlings caused by low temperature and enhance most of early maturity due to high temperature during reproductive stage with short duration available for the expression of various phenophases particularly the process of grain filling in case of very late sown crop. It might be probable reason for reduced number of ears/m^2, grains/ear and grain size compared to timely sowing. The reason for increased number of grains and its size was the most sensitive to temperature.

Stone and Nicolas (1995) reported the genotypic difference of grains/spike in response to high temperature. High temperature may affect the pollen viability and fertilization and thereby reduce the number of grains/spike. Individual grain weight which is considered as one of the major yield contributor was also significantly influenced by temperature.

Macas *et al.* (2000) studied the tolerance of durum wheat to high temperature during grain filling in an experiment in south Portugal. Nine durum (*Triticum durum*) and eight bread wheat (*Triticum aestivum*) genotypes were exposed to two different sowing date's i. e. normal and late. It was observed that grain yield and individual grain weight were significantly affected by increase in temperature.

Shahzad *et al*. (2002) found that the decrease in plant height in late sowing was due to shorter growing

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period. Early sown crop may have enjoyed the better environmental conditions especially the temperature and solar radiation which resulted to tallest plants.

Swilam *et al*. (2004) the analysis was carried out in two directions. The first one is to predict wheat yield under different sowing dates and the second one is to predict wheat yield under different growth stages at these sowing dates. Weather parameters for twenty-five years (1975-1999) were used to develop one prediction equation for each growth stage to predict wheat yield under the three sowing dates. In addition, weather parameters of 1999-00 season were used to predict wheat yield for the same year, then estimates were compared to actual yield and percentage decrease in yield (PD%) was calculated. In addition, the yield of 2000-01 and 2001-02 were predicted using previous year weather parameters, and then estimates were compared to actual yields. For sowing wheat on 15 November, any of the four studied stages (end of tillering, booting, flowering and grain filling) was suitable to predict yield (PD% values were between 4.41 and 7.55). The most suitable stage to predict wheat yield when sowing was on 1 December, by the end of grain filling stage (PD% was 8.60%). When sowing was performed on 15 December, the most suitable stage was at the end of flowering (PD% was 11.36%). The lowest percentage decrease was obtained for the growing season of 2001-02 when wheat was sown on 15 November (2.55%), followed by the 2000-01 season at the same sowing date (7.46%). This is an indication that using previous year weather parameters to predict the following year yield was more accurate when wheat was sown on 15 November. The percentage decrease in yield as a result of delayed sowing was 3.37%, whereas the reduction was 8.24% when sowing was delayed until 15 December. It is recommended to finish land preparation and wheat sowing not later than 1 December or else yield reduction would occur.

Rahman *et al*. (2005) reported that higher temperature (30/20°C, day/night) induced significant reduction of final grain weight, grain growth rate and grain growth duration of wheat as compared to optimum temperature (25/15°C, day/night).

Marcellous and Singh (1972) found that the variation in yield of agricultural crop is largely due to changes in the weather parameters during the growing period. Temperature plays an important role during the vegetative growth and grain formation of wheat crop.

Rajput and Varma (1994) concluded based on field trials conducted during rabi (winter) seasons of 1983-85 at Morena, Madhya Pradesh that sowing wheat on 18-20 Nov. (normal) gave higher grain yields than late (2-5 Dec.) or very late (18-20 Dec.) sowing. of 10 cultivars tested, HD 2382 was suited to all sowing dates. Hundal (2004) observed that a 2°C increase in temperature in wheat or rice resulted in 15-17 percent decrease in grain yield of both crops but beyond that, the decrease was very high in wheat.

MATERIAL AND METHOD

Experimental Detail

The experiment consisting of 5 date of sowing and 4 wheat varieties were laid out in a randomized block design with three replications.

Irrigation

Six irrigations (excluding rainfall) were given to the crop for proper growth and development from sowing to maturity. Come up irrigation was given just after sowing and the rest five irrigations were given at crown root initiation, tillering, late jointing, flowering and dough stages, respectively.

Thermal sensitivity

The thermal stress is mainly expressed through the maturity period by a crop. For evaluation of thermal sensitivity of the varieties the following index has been used, table 1.

$$TSI = \frac{\text{Range of duration to maturity}}{\text{Average duration}} \times 100$$

Table 1. Categorization of the effect of thermal stress based on TSI

TSI Value	Thermal Tolerance
<5	Tolerant
5.1 – 10.0	Moderately Tolerant
10.1 –15.1	Moderately Susceptible
>15.1	Susceptible

Influence of weather parameters

The influence of different weather parameters on growth and yield was worked out through step wise regression analysis.

Statistical analysis

All the data were tabulated and analysed statistically as per the procedure suggested by Panse and Sukhatme (1967) and Chandel (1984). The F test was used for judging the significance of the treatment mean at 5% level. Whenever F test showed significant difference the differences between treatment means were further tested by using critical difference (CD) value.

To compare different mean value of treatments, critical difference (CD) values were calculated as follows:

$$SEm \pm = \sqrt{\frac{Ems}{n}}$$

(i)

Where,

SEm ± = Standard error of mean

Ems = Error mean square

n = Number of observations on which the mean values is based

(ii) CD (5%) = SEm x√ 2 x t at 5 % for Error d.f. (2.02).

Regression equation were also worked out to find out the differet weather parameters on grain yield.

RESULT AND DISCUSSION

Temperature during growth stages

Data regarding maximum, minimum and mean temperature during different Phenological stages under different thermal environment and genotypes are summarized in table 2.

Data presented in Table 2 reveals that wheat variety Kanchan sown on 25 November, 2009 experienced 27.5 °C maximum, 11.1 °C minimum and 19.3 mean temperature for emergence, 28.4°C, 12.9°C and 20.6°C temperature for CRI stage, 26.4°C, 11.0°C and 18.7°C for 50% flowering and 32.3°C, 16.3°C and 24.3°C for maturity stage during crop growth period. The difference in temperature at maturity stage from D_1 to D_5 for Kanchan was 6.8°C maximum, 5.2°C minimum and 6.0°C mean temperature.

Wheat variety GW-273 sown on 25 November, 2009 experienced 27.5°C maximum, 11.1°C minimum and 19.3 mean daily temperature for emergence, 28.4°C, 12.9°C and 20.6°C for CRI stage, 26.5°C, 11.0°C and 18.7°C for 50% flowering stage and 32.3°C, 16.3°C and 24.3°C for maturity stage. The difference in temperatures during maturity stage for variety GW-273 was 6.9 °C maximum, 5.3°C minimum and 6.1°C mean temperature from D_1 to D_5.

Sujata sown on D_1 (25 November, 2009) recorded 27.5°C maximum, 11.1°C minimum and 19.3 mean daily temperature for emergence, 28.4°C, 12.9°C and 20.6°C for CRI stage, 26.5°C, 11.1°C and 18.8°C for 50% flowering stage and 34.1°C, 17.5°C and 25.8°C for maturity stage during crop growth period. The difference in temperature from D_1 to D_5 for variety Sujata was 6.3 °C maximum, 4.6°C minimum and 5.4°C mean temperature.

Wheat variety Amar D_1 which (25 November, 2009) recorded 27.5°C maximum, 11.1°C minimum and 19.3 mean daily temperature for emergence, 28.4°C, 12.9°C and 20.6°C for CRI stage, 26.5°C, 11.1°C and 18.8°C for 50% flowering and 34.1°C, 17.5°C and 25.8°C for maturity stage during crop growth period. The difference in temperature under maturity stage for variety Amar was 6.4°C Maximum, 4.8°C Minimum and 5.6°C Mean temperature from D_1 to D_5.

In general the maximum, minimum as well as mean temperature was higher when the crop was sown on 05 December closely followed by 15 December, 25 November, 25 December and 05 January during sowing to emergence.

During emergence to CRI stage the maximum temperature was higher during 25 November sowing which gradually decreased when the sowing was delayed. Whereas, the minimum temperature was higher at 05 December sowing and mean temperature was higher at 25 November and 05 December sowing. The maximum, minimum as well as mean temperature increased gradually when the sowing was delayed from 25 November to 05 January CRI to 50% flowering and 50% flowering to maturity. At 50% flowering to maturity the maximum, minimum and mean temperature was observed as higher as 40.5, 22.3 and 31.4°C for variety Amar when sown on 05 January. This showed that 34-35°C maximum, 17-18°C minimum and 26-27°C mean temperature were more favorable for higher yield of wheat crop under

Temperature pattern

The growth and development of wheat crop apart from being governed by genetic characters depend largely on a number of environmental factors which very under different sowing dates (Saini *et al*., 1988). The sowing of wheat is delayed either to fit it in multiple or relay crop sequence where wheat is to be sown after a very short duration winter (Rabi) crop, or after long duration rice crop or sugarcane ratoon. The yield of wheat decreases with delayed sowing thought the magnitude of reduction varies with the varieties.

The patterns of maximum, minimum and mean temperature during different periods of wheat growth are shown in table 3, 4, 5 and 6. The data on temperature pattern revealed that the maximum and mean temperature was lower when the crop was sown on 25 December while the minimum temperature was lower on 05 January sowing as compared to other sowing dates from sowing to 30 days after sowing, fig 1.

During 31-40 DAS the maximum, minimum as well as mean temperature was lower on 15 December sowing, while they were lower on 05 December sowing during 41-50 DAS as compared to other sowing dates. After 51 DAS till maturity the lower values of maximum, minimum and mean temperature were recorded on 25 November sowing which increased gradually when the sowing was delayed and reached as high as 32.5°C and 32.6°C at maturity when the crop was sown on 05 January in different varieties. Singh *et al*. (2008) reported that maximum, temperature in the range 17-19°C during January and less than 20°C during February and 28-30°C during March were the most favorable for better wheat yield. However, the maximum temperature more than 21°C, 24°C and 30°C during the month of January, February and March respectively, resulted in lowest yield. On the other hand, minimum temperature in the range of 6-8°C in January more than 9°C in February and 13°C in March were found favorable for yield whereas

minimum temperature less than 4°C, 5°C and 11°C during the month of January, February and March respectively, were less favorable in south-western region of Punjab. Similar results were reported by Chaurasia *et al.*, (1995) for the central Punjab region.

Table 2. Effect of different thermal environments on phenology of wheat varieties

Sowing dates	Crop growth stages							
	Emergence	C.R.I.	Tillering	Ear emergence	50% Flow.	milking	Dough	maturity
Kanchan								
D_1-25 Nov.	5	22	34	51	68	89	100	110
D_2-05 Dec.	5	22	34	52	69	86	98	107(3)
D_3-15 Dec.	6	21	33	48	66	82	95	100(10)
D_4-25 Dec.	6	20	32	48	65	78	91	98(12)
D_5-05 Jan.	7	19	30	48	64	76	88	93(17)
GW-273								
D_1-25 Nov.	5	22	34	50	69	89	100	108
D_2-05 Dec.	5	22	33	50	67	86	98	105(3)
D_3-15 Dec.	6	21	33	49	68	82	95	100(8)
D_4-25 Dec.	6	20	32	48	66	78	91	98(10)
D_5-05 Jan.	7	19	30	48	63	76	89	95(13)
Sujata								
D_1-25 Nov.	5	22	35	64	74	94	106	118
D_2-05 Dec.	5	22	34	64	76	92	100	111(7)
D_3-15 Dec.	5	21	34	62	74	87	98	105(13)
D_4-25 Dec.	6	20	33	61	69	80	95	100(18)
D_5-05 Jan.	6	19	31	60	68	78	92	98(20)
Amar								
D_1-25 Nov.	5	22	36	65	74	94	106	118
D_2-05 Dec.	5	22	35	64	77	92	100	111(7)
D_3-15 Dec.	5	21	34	63	74	87	98	105(13)
D_4-25 Dec.	6	21	34	63	70	80	95	100(18)
D_5-05 Jan.	6	20	32	61	69	78	92	98(20)

Figures in parenthesis are decrease in duration as compared to D_1

Table 3. Difference in maximum, minimum and mean temperatures (°C) of different heat varieties from D_1 to D_2, D_3, D_4 and D_5 at 50% flowering to maturity

Variety	Difference in temperatures (^{0}C) From D_1 to			
	D_2-05 Dec.	D_3-15 Dec.	D_4-25 Dec.	D_5-05 Jan.
		Maximum Temperature		
Kanchan	2.1	3.2	5.3	6.8
GW-273	1.7	3.5	5.5	6.9
Sujata	1.6	3.1	4.1	6.3
Amar	1.8	3.1	4.1	6.4
		Minimum Temperature		
Kanchan	1.6	1.9	4.2	5.2
GW-273	1.4	2.2	4.4	5.3
Sujata	0.8	2.5	3.7	4.6
Amar	1.0	2.5	3.8	4.8
		Mean Temperature		
Kanchan	1.9	2.6	4.8	6
GW-273	1.6	2.9	5.0	6.1
Sujata	1.2	2.8	3.9	5.4
Amar	1.4	2.8	4.0	5.6

Table 4. Pattern of maximum temperature during different periods

Sowing dates	Crop growth stages							
	0-30	31-40	41-50	51-60	61-70	71-80	81-90	91-Maturity
Kanchan								
D_1-25 Nov.	27.7	25.9	26.9	25.6	28.2	29.0	31.6	34.6
D_2-05 Dec.	27.3	26.9	25.6	28.2	29.0	31.6	34.0	36.5
D_3-15 Dec.	26.5	25.6	28.2	29.0	31.6	34.0	35.3	39.1
D_4-25 Dec.	26.1	28.2	29.0	31.6	34.0	35.3	39.1	40.5
D_5-05 Jan.	27.0	29.2	31.7	34.3	35.4	39.4	40.5	41.6
GW-273								
D_1-25 Nov.	27.7	25.9	26.9	25.6	28.2	29.0	31.6	34.7
D_2-05 Dec.	27.3	26.9	25.6	28.2	29.0	31.6	34.0	36.2
D_3-15 Dec.	26.5	25.6	28.2	29.0	31.6	34.0	35.3	39.1
D_4-25 Dec.	26.1	28.2	29.0	31.6	34.0	35.3	39.1	40.5
D_5-05 Jan.	27.0	29.2	31.7	34.3	35.4	39.4	40.5	41.7

Sujata								
D_1-25 Nov.	27.7	25.9	26.9	25.6	28.2	29.0	31.6	35.7
D_2-05 Dec.	27.3	26.9	25.6	28.2	29.0	31.6	34.0	37.3
D_3-15 Dec.	26.5	25.6	28.2	29.0	31.6	34.0	35.3	39.5
D_4-25 Dec.	26.1	28.2	29.0	31.6	34.0	35.3	39.1	40.4
D_5-05 Jan.	27.0	29.2	31.7	34.3	35.4	39.4	40.5	42.3
Amar								
D_1-25 Nov.	27.7	25.9	26.9	25.6	28.2	29.0	31.6	35.7
D_2-05 Dec.	27.3	26.9	25.6	28.2	29.0	31.6	34.0	37.3
D_3-15 Dec.	26.5	25.6	28.2	29.0	31.6	34.0	35.3	39.5
D_4-25 Dec.	26.1	28.2	29.0	31.6	34.0	35.3	39.1	40.4
D_5-05 Jan.	27.0	29.2	31.7	34.3	35.4	39.4	40.5	42.3

Table 5. Pattern of mean temperature during different periods

Sowing dates	**Crop growth stages**							
	0-30	**31-40**	**41-50**	**51-60**	**61-70**	**71-80**	**81-90**	**91-Maturity**
Kanchan								
D_1-25 Nov.	20.3	18.4	19.4	17.7	19.0	21.2	24.0	26.4
D_2-05 Dec.	20.0	19.4	17.7	19.0	21.2	24.0	25.2	28.1
D_3-15 Dec.	19.3	17.7	19.0	21.2	24.0	25.2	27.6	29.4
D_4-25 Dec.	18.5	19.0	21.2	24.0	25.2	27.6	29.4	32.5
D_5-05 Jan.	18.8	21.4	24.1	25.7	27.4	29.9	32.4	31.7
GW-273								
D_1-25 Nov.	20.3	18.4	19.4	17.7	19.0	21.2	24.0	26.4
D_2-05 Dec.	20.0	19.4	17.7	19.0	21.2	24.0	25.2	28.0
D_3-15 Dec.	19.3	17.7	19.0	21.2	24.0	25.2	27.6	29.4
D_4-25 Dec.	18.5	19.0	21.2	24.0	25.2	27.6	29.4	32.5
D_5-05 Jan.	18.8	21.4	24.1	25.7	27.4	29.9	32.4	32.2
Sujata								
D_1-25 Nov.	20.3	18.4	19.4	17.7	19.0	21.2	24.0	27.1
D_2-05 Dec.	20.0	19.4	17.7	19.0	21.2	24.0	25.2	28.6
D_3-15 Dec.	19.3	17.7	19.0	21.2	24.0	25.2	27.6	30.4
D_4-25 Dec.	18.5	19.0	21.2	24.0	25.2	27.6	29.4	32.3
D_5-05 Jan.	18.8	21.4	24.1	25.7	27.4	29.9	32.4	32.6
Amar								
D_1-25 Nov.	20.3	18.4	19.4	17.7	19.0	21.2	24.0	27.1
D_2-05 Dec.	20.0	19.4	17.7	19.0	21.2	24.0	25.2	28.6
D_3-15 Dec.	19.3	17.7	19.0	21.2	24.0	25.2	27.6	30.4
D_4-25 Dec.	18.5	19.0	21.2	24.0	25.2	27.6	29.4	32.3
D_5-05 Jan.	18.8	21.4	24.1	25.7	27.4	29.9	32.4	32.6

Table 6. Pattern of minimum temperature during different periods

Sowing dates	**Crop growth stages**							
	0-30	**31-40**	**41-50**	**51-60**	**61-70**	**71-80**	**81-90**	**91-Maturity**
Kanchan								
D_1-25 Nov.	12.9	10.9	11.9	9.8	9.7	13.4	16.5	18.2
D_2-05 Dec.	12.8	11.9	9.8	9.7	13.4	16.5	16.5	19.8
D_3-15 Dec.	12.0	9.8	9.7	13.4	16.5	16.5	19.9	19.8
D_4-25 Dec.	10.9	9.7	13.4	16.5	16.5	19.9	19.8	24.5
D_5-05 Jan.	10.6	13.6	16.5	17.1	19.4	20.3	24.2	21.9
GW-273								
D_1-25 Nov.	12.9	10.9	11.9	9.8	9.7	13.4	16.5	18.1
D_2-05 Dec.	12.8	11.9	9.8	9.7	13.4	16.5	16.5	19.7
D_3-15 Dec.	12.0	9.8	9.7	13.4	16.5	16.5	19.9	19.8
D_4-25 Dec.	10.9	9.7	13.4	16.5	16.5	19.9	19.8	24.5
D_5-05 Jan.	10.6	13.6	16.5	17.1	19.4	20.3	24.2	22.6
Sujata								
D_1-25 Nov.	12.9	10.9	11.9	9.8	9.7	13.4	16.5	18.6
D_2-05 Dec.	12.8	11.9	9.8	9.7	13.4	16.5	16.5	19.9
D_3-15 Dec.	12.0	9.8	9.7	13.4	16.5	16.5	19.9	21.4
D_4-25 Dec.	10.9	9.7	13.4	16.5	16.5	19.9	19.8	24.2
D_5-05 Jan.	10.6	13.6	16.5	17.1	19.4	20.3	24.2	22.9
Amar								
D_1-25 Nov.	12.9	10.9	11.9	9.8	9.7	13.4	16.5	18.6
D_2-05 Dec.	12.8	11.9	9.8	9.7	13.4	16.5	16.5	19.9
D_3-15 Dec.	12.0	9.8	9.7	13.4	16.5	16.5	19.9	21.4
D_4-25 Dec.	10.9	9.7	13.4	16.5	16.5	19.9	19.8	24.2
D_5-05 Jan.	10.6	13.6	16.5	17.1	19.4	20.3	24.2	22.9

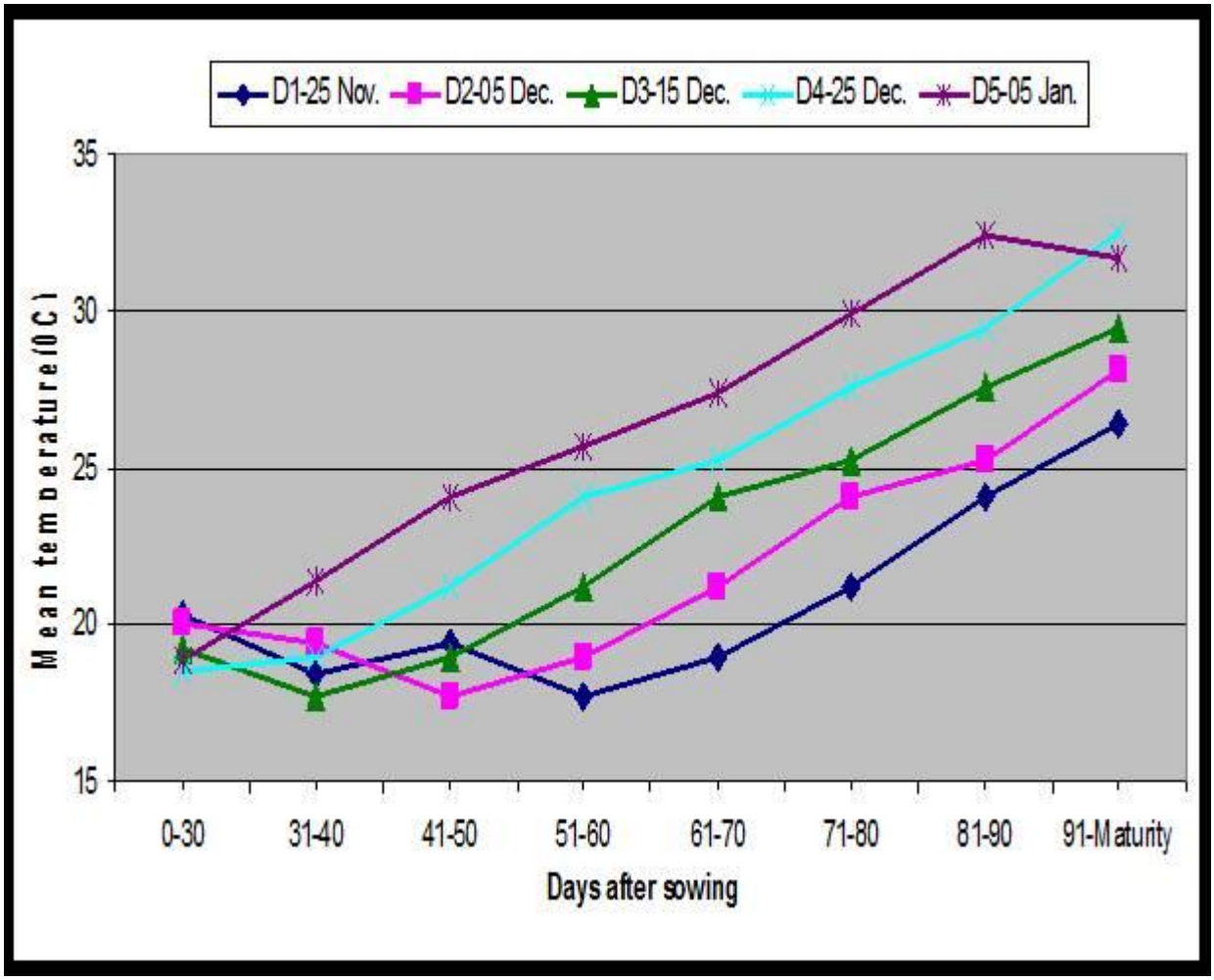
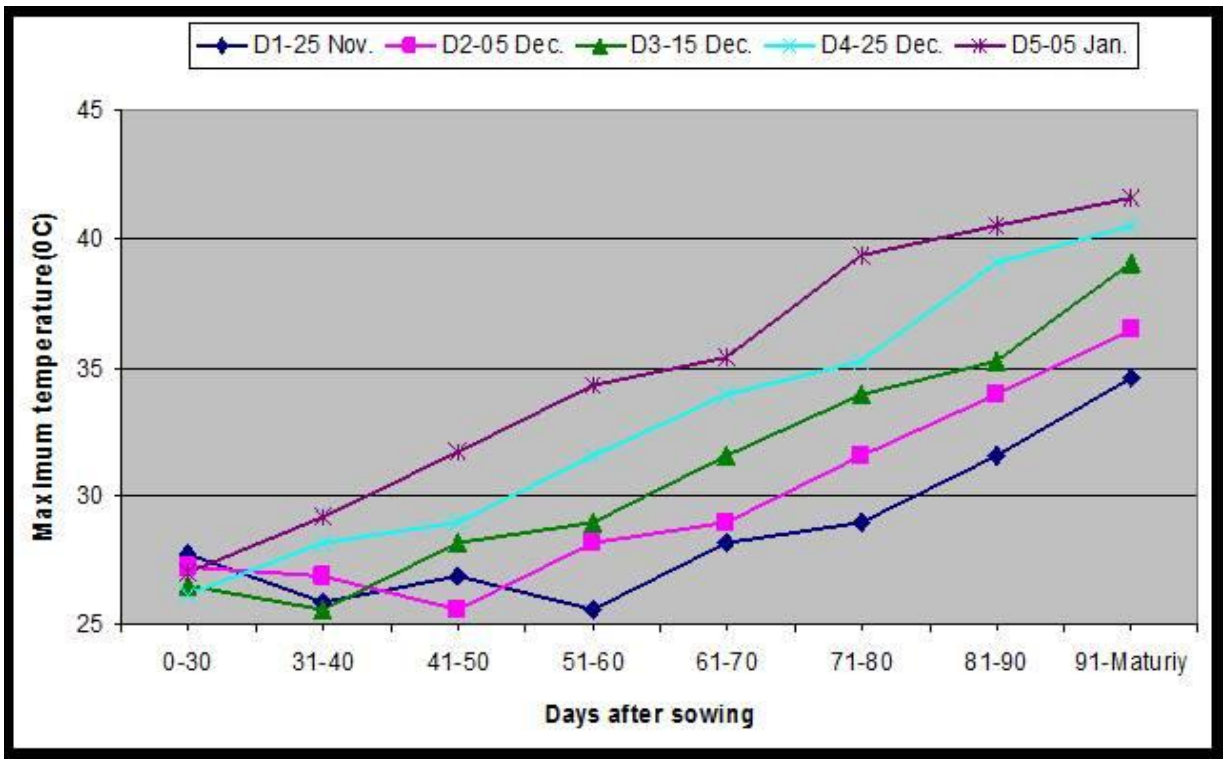


Fig. 1. Pattern of Mean temperature during sowing to maturity period under different environments (D1 to D5)



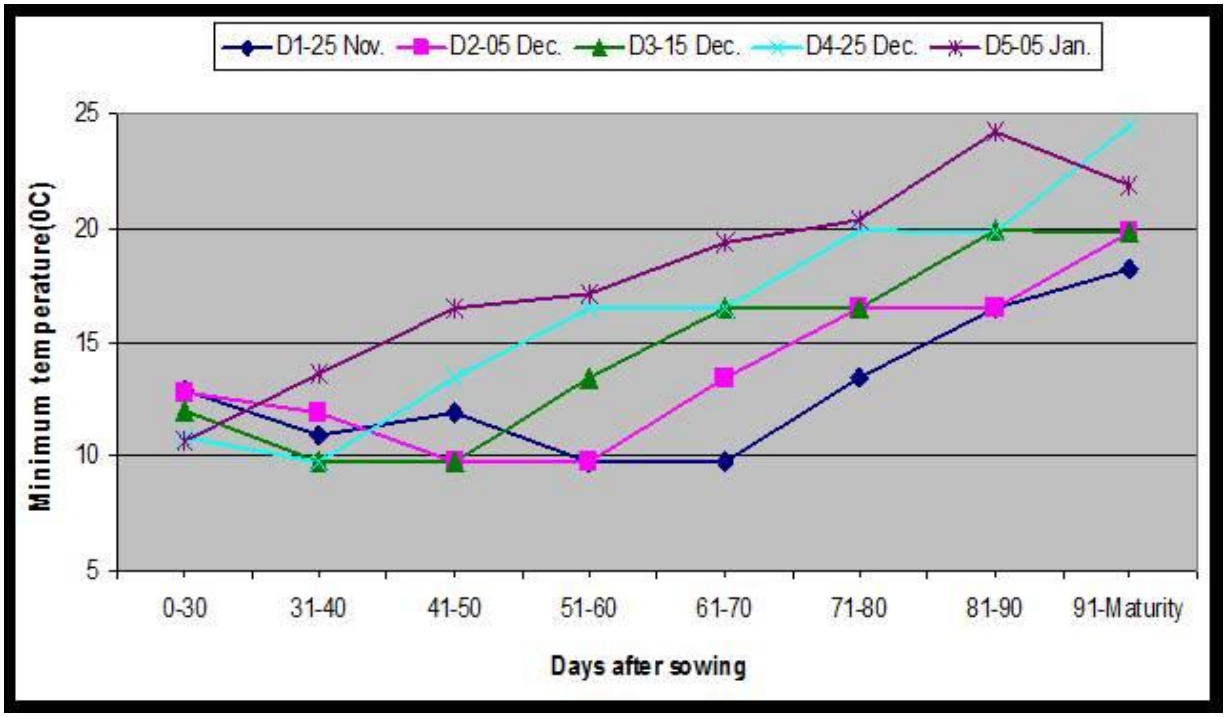


Fig. 2. Pattern of Maximum and Minimum temperatures during sowing to maturity periods under different environments (D1 to D5)

CONCLUSION

It can be seen that the highest effect of thermal stress on phonological stages was observed in cultivar Sujata and Amar followed by Kanchan. The least effect of thermal stress was observed in case of GW-273 (13 days). The variety GW-273 was not affected much due to thermal stress and the duration decreased only by 10 and 13 days respectively under 25 December and 05 January sowing. The maximum, minimum as well as mean temperatures increased gradually when the sowing was delayed from 25 November to 05 January CRI to 50% flowering and 50% flowering to maturity. At 50% flowering to maturity the maximum, minimum and mean temperatures was observed as higher as 40.5, 22.3 and 31.4°C for variety Amar when sown on 05 January. This showed that 34-35°C maximum, 17-18°C minimum and 26-27°C mean temperature were more favorable for higher yield of wheat crop under Raipur condition. The patterns of maximum, minimum and mean temperatures during 31-40 DAS the maximum, minimum as well as mean temperatures was lower on 15 December sowing while they were lower on 05 December sowing during 41-50 DAS as compared to other sowing dates. After 51 DAS till maturity the lower values of maximum, minimum and mean temperature were recorded on 25 November sowing which increased gradually when the sowing was delayed and reached as high as 32.5°C and 32.6°C at maturity when the crop was sown on 05 January. Length of ear of different wheat varieties was influenced due to temperature and shifting thermal environment. Longer ear head (9.4 cm) was observed in first and second date of sowing (25 November and 05 December) as compared to delayed sowing of 15 December, 25 December and 05 January. Longer ear head was observed in variety GW-273 (9.4) while minimum (8.4) was observed in Amar. Number of grain/ear head with 05 December sowing was found next that of 25 November sowing but significant superior over 15 and 25 December sowing. Significantly lower number of grains/ear head was observed with 05 January sowing. Among different variety GW-273 produced significantly higher number of grains/ear head closely followed by Kanchan being at par to each other (50 and 48, respectively) Sujata and Amar produced lower number of grains/ear head and were found statistically similar to each other.

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RESEARCH ARTICLE

EVALUATION OF DIFFERENT CUCUMBER STRAIN FOR VARIOUS HORTICULTURAL TRAITS UNDER VALLEY CONDITION OF GARHWAL HIMALAYA

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Abstract: The present research was undertaken with 14 different strains of cucumber for evaluating their ability for various quantitative and qualitative horticultural traits under Garhwal Himalaya Region. The analysis of varience reaveled highly significant for all the characters studied. The K-90 recorded highest vine length (310.59 cm), number and T.S.S (6.84 °Brix). Whereas HP-2 recorded minimum days taken to opening of 1st female flower (43.21) maximum % of fruit setting (93.40), number of fruits/vine (20.00), and carbohydrate (3.39). SPP-63 showed minimum number of nodes bearing first male flower (4.25) and days taken to opening of 1st male flower (40.23). The strain New Manipur-1 recorded maximum number of primary branches/plant (12.23), minimum sex ratio (10:1), average fruit weight (205.05 g), fruit diameter (6.59), fruit yield/vine (3.61 kg), fruit yield/plot (44.46 kg), fruit yield/ha. (49.42 t/ha.), vitamin C (7.63 mg/100g) and minimum number of nodes bearing first female flower (6.11) and Maximum strains used in this research work are superior in different characters, which could be use for the improvement programmes.

Keywords: Cucumber, Quantitative, Qualitative, Sex, Fruit, Yield

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the important crops of cucurbitaceous vegetable group from their nutritional as well as economic point of view. It is an ideal summer vegetable crop mostly grown for its edible tender fruits, preferred as salad ingredient, pickles and as a cooked vegetable. Cucumber has got cooling effect, so in the eastern countries; fruits are often used as cooling vegetable. It is ideal for people suffering from jaundice and allied diseases and also very much useful in preventing constipation. Seeds contain oil, which is helpful for brain development and body smoothness. For quick symptomatic cooling of the head, hand and feet, cucumber paste when applied on the body part is very effective in bringing a refrigerator effect. It may further be mentioned that cucumber juice is commonly used for treating diseases of teeth and gums. Its juice is still useful for rheumatic conditions and healthy growing of hair (Khulakpam *et al.*, 2015). Hence, it is being used in Ayurvedic preparations (Decker-Walter, 1999). Besides this, the whole fruit is used in cosmetic and soap industries. Cucumber originated in Northern India and has been in cultivation for at least 3000 years. From India, it spread to China, Asia Minor, North Africa and Southern Europe. Now, it is extensively cultivated in tropics, sub-tropics and in middle region of temperate zone. Cucumber (*Cucumis sativus* L.) is one of the oldest amongst the cultivated vegetable crops and has been found in cultivation since 3000 to 4000 years. Biochemically the cucurbits are characterized by bitter principles, called cucurbitacins *i.e.* tetracyclic triterpenes (Jeffery, 1983). Majority of the cucurbits are either monoecious or andromonoecious (a few dioecious) with trailing habit and are pollinated by insects. It is one of quick maturing vine vegetables crops. However it can be grown in both summer and rainy season. India is being native place of cucumber possesses vast genetic variability for vegetative and fruit characters. Low fruiting ability and yield suppression due to its inherent fruiting habits are major factors limiting fruit yield in slicing and processing cucumber. The yield and quality of crop are very complex characteristics. Cucumber is a monoecious crops but its bear's hermaphrodite flowers also. Sex of cucumber is highly influence by climatic condition and nutritional level of soil. Cucumber produces male flower if the nutrition level of soil and temperature is high due to high vegetative growth. The temperature and nutrition level is low, plant producing maximum number of female flowers. So that characteristics of a cultivar as well as combination of traits differ according to climatic conditions of the localities. At present, urgent need of the farmers/ scientist is to develop early maturing and high yielding variety/ hybrid. Preliminary identification of early maturing genotypes can be done based on characters like days to opening of female flowers, node number to first female flowering and days to fruit picking. Collection and evaluation of germplasm is a pre-requisite for their utilization. Therefore, a trial for characterization and evaluation of presently available cucumber germplasm is carried out in order to identify the potential cultivar for different horticultural characters. The present investigation was designed for a comparative study of cucumber genotypes, which is suitable for valley condition of Garhwal Himalaya region.

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MATERIAL AND METHOD

The field trial was conducted at Horticultural Research Center, Chauras Campus, H.N.B Garhwal University, Srinagar (Garhwal), Uttarakhand during Zaid season, in 2014-2015. Srinagar (Garhwal) is located in the Alaknanda valley (78° 47' 30" E longitude and 30° 13' 0" N latitude and at an elevation of 540 m above msl), a semi-arid, subtropical climate with dry summer and rigorous winters. For present research work the research materials were collected from IIVR, Varanasi, Manipur and Uttarakhand *viz.* PB-Naveen, K-90, Swarna Purna, Seven Star, SPP-63, New Manipur-1, New Manipur-2, GP-1, HP-1, HP-2, Mandal, RAJ-1, RAJ-2 and Pusa Sanyog. The experimental plot was ploughed thrice and bought to fine tithe and then levelled. The experiment trial was carried out in Randomized Block Design with three replications. The whole area of experimental site was divided into three blocks of equal size and each block possessed 14 plots. Each plot measured 4.50 x 2.0 m^2 area. The seedling were transplanting at four leaf stage *i.e.*, 25-30 days after sowing. Transplanting of seedlings was done in evening hours in each experimental plot at the spacing of 1.50 X 0.50 cm, according to experimental model. After transplanting, a light irrigation was given for the proper establishment of the seedlings. Farmyard manure was applied at the rate of 25 tonnes/ ha and NPK fertilizer applied in the rate of 80:60:60 kg/ha respectively. Full does of phosphorus, potassium and half does of nitrogen were applied at the time of transplanting, remaining half does of nitrogen was applied at 35days after transplanting. All the cultural activities and plant protection measures recommended for the successful crop. Five plants were selected randomly from each plot for recording the data on the following observations *viz.*, vine length (cm), number of nodes/ plant, number of primary branch/ plant, leaf area (cm^3), days taken to opening of first male flower, days taken to opening of first female flower, number of nodes bearing first male flower, number of nodes bearing first female flower, sex ratio, percent of fruit setting, fruit length (cm), fruit diameter (cm), number of locules/fruit, yield/plant (kg), yield/plot (kg), fruit yield/ hectare (q), harvesting duration of crop, TSS (°Brix), carbohydrate (g/100g), Vit. A (mg/100g), Vit.C (mg/100g), phosphorus (mg/ 100g) and calcium (mg/100g). The obtained data were statistically analyzed according to the procedure of R.B.D. as stated by Panse and Sukhatme (1967). The significance of variation among the treatments was observed by applying ANOVA and critical difference at 1% level (CD) was calculated to compare the mean values of treatments for all the characters.

RESULT AND DISCUSSION

The analaysis of variance observed highly significant for all the quantitative and qualitaive traits under studied. The experimental results showed that the maximum strains were differences for quantitative and qualitative traits.

Growth characters

The experimental results as shown in table 1 observed that among all the strains, K-90 showed maximum vine length (310.59 cm) followed by SPP-63 (305.49 cm), HP-2 (303.48 cm) and minimum vine length was recorded in New Manipur-2 (192.39 cm). The variation in vine length might be due to genetic reasons of different strains, inherent properties, hormonal and enzymatic response of plants, environmental factors and nutritional properties of soil. Similar results have also been reported by Solanki and seth (1980) in cucumber. Maximum number of primary branches/vine was recorded in New Manipur-1 (12.23) followed by HP-2 (11.73) and Mandal (11.15) while, the minimum number of branches per vine was recorded in HP-1 (7.90). The variation in number of primary branches/ vine might have been due to vine length and number of nodes in plants, because the primary branches rise from nodes of plants and environmental factors play a key role in primary branches emergence which is confirming to reports of Sharma and Bhattarai (2006) in cucumber. Intermodal length play the main role in primary branches and flower appearances because each primary branches and flowers emergence near to internodes. The minimum intermodal length was recorded in RAJ-2 (4.94 cm) followed by HP-2 (5.30 cm) and Pusa Sanyog (5.61 cm) where as maximum intermodal length was observed in SPP-63 (8.49 cm). The flowering stage is one of the key factors that decides the earliness and lateness of crop production. Minimum days to first appearance of male flower was observed in SPP-63 (40.23 days) followed by HP-2 (41.28 days) and Seven Star (42.10 days). Maximum days to first appearance of male flower were found in K-90 (50.71 days). Minimum days taken to first female flower appearances were observed in HP-2 (43.21 days) followed by SPP-63 (44.15 days) and New Manipur-2 (44.50 days). Maximum days were recorded to first appearance of female flower in RAJ-1 (58.69 days). The number of days from first appearance of female flower is an important character that indicates earliness or lateness of the crop. The variation in first appearance of male and female flower might have been due to genetic nature of crop, hormonal balance, crop vigour, soil fertility and environmental factor. Similar results have been reported by Sahni *et al.*, 1987 in ridge gourd, Badgurjar and More (2004) and Bairagi *et al.* 2005 in cucumber. Minimum number of node at which first male flower appeared were observed in SPP-63 (4.25) followed by Pusa Sanyog

(4.33) and HP-2 (4.48). Maximum number of node at which first male flower was appeared in PB-Naveen and New Manipur-2 (6.50) respectively. Minimum number of node at which first female flower appeared was recorded in New Manipur-1 (6.11) followed by HP-1 (6.29) and SPP-63 (6.30). Maximum number of node at which first female flower appeared was in Pusa Sanyog (8.66). The variation in number of node at which first male and female flower appears might have been due to inherent properties of strains that's highly influences by environmental conditions. Similar results have been reported by Bairagi *et al.,* 2005 and Sharma and Bhattarai (2006) in cucumber.

Yield characters

Data presented in Table 2 revealed that the variation in sex ratios (number of male and female flowers) per vine was influence by genetic factors, high vegetative growth, hormonal balance and environmental factors. Minimum sex ratio was recorded in New Manipur-1 (10:1) followed by Mandal (12:1) and K-90 (13:1). The maximum sex ratio was observed in Pusa Sanyog (21:1). Similar results have been reported by Solanki and Seth (1980), Rastogi *et al.,* 1990 and Bairagi *et al.,* 2005 in cucumber. Highest percent of fruit setting was recorded in HP-2 (93.40 %) followed by RAJ-1 (91.24 %) and PB-Naveen and Swarna Purna (90.22%) respectively, whereas Pusa Sanyog (78.61 %) showed minimum fruit setting. The fruit setting is depends on the good pollination, favorable climatic conditions, genetic factors and soil fertility conditions. Minimum days to first fruit harvest was recorded in New Manipur-1 (52.43 days) followed by SPP-63 (55.85 days) and Seven Star (58.24 days). RAJ-2 (74.66 days) had taken very much time to first fruit harvesting. The variation in days to first fruit harvesting might have been due to genetic factor, environmental factor, hormonal factor and vigour of the crop. The average fruit weight and number of fruit/ plant were highly influences the fruit yield/ plot and fruit yield/ hectare. The maximum number of fruit/ vine was recorded in HP-2 (20.00 fruits) followed by RAJ-1 (19.35 fruits) and RAJ-2 and pusa Sanyog (18.79 fruits) respectively. The lowest number of fruit/ plant was recorded in SPP-63 (12.34 fruits). The number of fruits/ vine is one of the major factors for deciding the yield of the crop. The variation in number of fruits per vine might have been due to sex ratio, fruit set percentage, genetic nature and their response to varying environmental and soil conditions. Variation in number of fruits per vine was also reported by Nag *et al.,* 2012 in ivy gourd and Srivastava and Srivastava (1976) in bitter gourd. The maximum average fruit weight was recorded in New Manipur-1 (205.05 g) followed by HP-1 (200.32 g) and SPP-63 (195.44g). Minimum fruit weight was recorded in Seven Star (145.35 g). Maximum fruit length was recorded in Swarna Purna (20.93 cm) followed by GP-1 (19.62 cm) and PB-Naveen (18.81 cm). The minimum fruit length was recorded in New Manipur-1 (14.52 cm). Maximum fruit diameter was recorded in New Manipur-1 (6.59 cm) followed by RAJ-2 (5.94 cm) and Mandal (5.88 cm). The minimum fruit diameter was found in K-90 (3.90 cm). The yield is directly influences by the length and diameter of fruits. The variation in fruit length and diameter might have been due to genetic factors and environmental factor Ahamed *et al.,* 2004 and Rastogi *et al.,* 1990 have also reported similar findings in cucumber. Maximum locules/fruit was recorded in Pusa Sanyog (6.00) followed by GP-1 (5.89) and K-90 (5.60) whereas minimum number of locules were recorded in New Manipur-2 (3.00). The maximum fruit yield/vine was recorded in New Manipur-1 (3.61 Kg) followed by RAJ-1 (3.27 Kg) and RAJ-2 (3.24 Kg) while, minimum fruit yield/vine was recorded in PB-Naveen (2.06 Kg). The maximum yield/ plot was recorded in New Manipur-1 (44.46 Kg) followed by RAJ-1 (39.42 Kg) and RAJ-2 (38.51 Kg). Minimum yield/ plot were recorded in PB-Naveen (24.66 Kg). The maximum yield/ hectare was recorded in New Manipur-1 (49.42 t/ ha) followed by RAJ-1 (43.74 t/ ha) and HP-1 (42.64 t/ha). Minimum yield/ hectare were recorded in PB-Naveen (27.29 t/ha).

Quality characters

Maximum T.S.S. was found in K-90 (6.84 °Brix) followed by RAJ-2 (6.80 °Brix) and PB-Naveen and Swarna Purna (6.00 °Brix) respectively. The minimum TSS value was found in New Manipur-1 (4.23 °Brix). The higher TSS value may be due to its inherent characteristics. Maximum carbohydrates was recorded in GP-1 and HP-2 (3.39 g/100g) followed by New Manipur-2 (3.23 g/100g) and RAJ-1 (3.20 g/100g). The minimum carbohydrate was found in K-90 (2.58 g/100g). Maximum vitamin C was found in New Manipur-1 and HP-1 (7.63 mg/100g) followed by Pusa Sanyog (7.57 mg/100g) and K-90 (7.50 mg/100g). The lowest vitamin C was found in PB-Naveen (6.76 mg/100g). Maximum calcium content was observed in Pusa Sanyog (17.29 mg/100g) followed by New Manipur-1 (17.27 mg/100g) and K-90 (17.19 mg/ 100g). The lowest calcium content was found in PB-Naveen (9.25 mg/ 100g). Maximum phosphorus was recorded in SPP-63 (29.54 mg/100g) followed by Swarna Purna and HP-1 (29.40 mg/100g) and Seven Star (29.00 mg/100g). The lowest phosphorus was found in Pusa Sanyog (21.14 mg/100g). All quality characters are mainly governed by inheritance of parents; they are not influence by environmental factor and soil conditions.

Table 1. Performance of fourteen Cucumber (*Cucumis sativus* L.) strain for growth characters.

Treatment	Plant height (cm)	No. primary branch/vine	Internodal length (cm)	Number of nodes bearing first male flower	Number of nodes bearing first female flower	Days taken to opening of 1st male flower	Days taken to opening of 1st female flower
T_1 (PB-Naveen)	297.49	10.18	6.50	6.50	8.00	50.43	55.24
T_2 (K-90)	310.59	8.54	6.75	5.73	8.58	50.71	54.62
T_3 (Swarna Purna)	270.44	8.64	5.68	5.93	7.96	42.61	47.69
T_4 (Seven Star)	265.42	10.32	8.28	6.01	8.54	42.10	47.20
T_5 (SPP-63)	305.49	9.82	8.49	4.25	6.30	40.23	44.15
T_6 (New Manipur-1)	300.49	12.23	7.62	6.00	6.11	44.85	47.34
T_7 (New Manipur-2)	192.39	9.97	5.92	6.50	8.50	42.61	44.50
T_8 (GP-1)	285.69	10.22	6.58	6.49	6.80	50.00	55.74
T_9 (HP-1)	298.32	7.90	7.55	6.30	6.29	43.66	47.57
T_{10} (HP-2)	303.48	11.73	5.30	4.48	6.96	41.28	43.21
T_{11} (Mandal)	301.64	11.15	7.01	6.21	7.13	44.17	50.72
T_{12} (RAJ-1)	198.45	7.93	8.35	5.41	7.46	49.53	58.69
T_{13} (RAJ-2)	195.47	8.29	4.94	5.78	7.32	49.28	58.24
T_{14} (Pusa Sanyog)	267.40	9.20	5.61	4.33	8.66	49.30	57.36
Sem±	0.664	0.024	0.201	0.074	0.028	0.314	0.044
CD 1%	1.942	0.070	0.587	0.218	0.082	0.918	0.130

Table 2. Performance of fourteen Cucumber (*Cucumis sativus* L.) strain for yield characters.

Treatment	Sex ratio	% of fruit setting	Days taken to 1st fruit harvesting	No. of fruits/ vine	Average fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	No. of locules/ fruit	Fruit yield/vine (kg)	Fruit yield/plot (Kg)	Fruit yield/ha. (t/ha)
T_1 (PB-Naveen)	15:1	90.22	73.62	13.81	150.34	18.81	4.18	5.00	2.06	24.66	27.29
T_2 (K-90)	13:1	79.52	74.29	13.88	168.56	16.19	3.90	5.60	2.40	28.16	31.54
T_3 (Swarna Purna)	17:1	90.22	59.72	14.58	180.64	20.93	4.80	4.00	2.65	31.59	35.07
T_4 (Seven Star)	15:1	79.40	58.24	14.37	145.35	15.43	5.62	5.20	2.08	25.07	27.82
T_5 (SPP-63)	20:1	90.18	55.85	12.34	195.44	18.30	4.85	5.25	2.42	28.84	32.05
T_6 (New Manipur-1)	10:1	89.52	52.43	18.52	205.05	14.52	6.59	4.00	3.61	44.46	49.42
T_7 (New Manipur-2)	15:1	86.20	60.50	13.18	189.31	15.34	5.35	3.00	2.46	29.71	32.74
T_8 (GP-1)	18:1	83.54	71.52	15.85	175.48	19.62	5.87	5.89	2.77	33.38	37.03
T_9 (HP-1)	16:1	80.76	60.28	15.61	200.32	18.09	5.13	5.45	3.17	38.42	42.64
T_{10} (HP-2)	14:1	93.40	59.25	20.00	150.52	18.55	4.45	5.00	3.03	36.13	40.12
T_{11} (Mandal)	12:1	80.21	60.67	16.33	175.33	16.41	5.88	3.25	2.83	33.88	37.60
T_{12} (RAJ-1)	19:1	91.24	73.45	19.35	170.21	17.61	4.37	5.00	3.27	39.42	43.74
T_{13} (RAJ-2)	20:1	85.62	74.66	18.79	170.83	15.42	5.94	4.00	3.24	38.51	37.23
T_{14} (Pusa Sanyog)	21:1	78.61	73.35	18.79	160.23	17.38	4.54	6.00	2.75	33.05	36.62
Sem±	0.038	0.036	0.087	0.035	0.548	0.101	0.035	0.519	0.024	0.070	0.084
CD 1%	0.124	0.106	0.255	0.102	0.249	0.296	0.102	1.517	0.069	0.204	0.246

Table 3. Performance of fourteen Cucumber (*Cucumis sativus* L.) strain for quality characters.

Treatment	TSS (°Brix)	Carbohydrate (g/100g)	Vitamin C (mg/100g)	Calcium (mg/100g)	Phosphorus (mg/100g)
T_1 (PB-Naveen)	6.00	2.81	6.76	9.25	21.27

T_2 (K-90)	6.84	2.58	7.50	17.19	22.63
T_3 (Swarna Purna)	6.00	2.74	7.31	15.74	29.40
T_4 (Seven Star)	5.79	2.88	7.07	13.82	29.00
T_5 (SPP-63)	5.51	2.59	6.89	9.45	29.54
T_6 (New Manipur-1)	4.23	3.10	7.63	17.27	28.20
T_7 (New Manipur-2)	4.65	3.23	7.35	10.18	21.28
T_8 (GP-1)	5.50	3.39	7.33	12.57	27.35
T_9 (HP-1)	4.89	3.16	7.63	9.57	29.40
T_{10} (HP-2)	5.89	3.39	7.21	16.84	21.52
T_{11} (Mandal)	5.50	2.91	6.89	14.57	23.61
T_{12} (RAJ-1)	5.56	3.20	6.87	13.20	24.81
T_{13} (RAJ-2)	6.80	2.99	6.83	12.64	25.56
T_{14} (Pusa Sanyog)	4.33	3.14	7.57	17.29	21.14
Sem±	0.119	0.108	0.077	0.026	0.065
CD 1%	0.348	0.315	0.225	0.077	0.191

CONCLUSION

From the above studies it may be concluded that different strain showed different good quantitative and qualitative traits. So, HP-2 showed superior for days taken to opening of 1st female flower, percent of fruit setting, number of fruits/vine and carbohydrate. And the strain New Manipur-1 was found superior for number of primary branch/vine, number of nodes bearing first female flower, sex ratio, average fruit weight, fruit diameter, fruit yield/vine, fruit yield/plot, fruit yield/ha. and vitamin C respectively. The strain New Manipur-1 and other strains could be used in future improvement programmes.

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RESEARCH ARTICLE

IMPACT OF ABIOTIC FACTORS ON THE DISEASE DEVELOPMENT OF ALTERNARIA BLIGHT OF CORIANDER CAUSING *ALTERNARIA POONENSIS* RAGUNATH

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Abstract: An investigation on Coriander susceptible cultivar Raunak-31 suffered from Alternaria blight caused by *Alternaria poonensis* Raghunath was conducted during *Rabi* October 2012 to February 2013. Five isolates were recovered from samples collected from Kota (1), Udaipur (3) and Baran (1). Of the three isolates of *A. poonensis* (Ap-01, Ap-02 and Ap-03) evaluated for pathogenic variability on pot -grown plants of susceptible cultivar Raunak-31, the maximum disease (53.2% PDI) was by isolate Ap-01and minimum (32.4%) by Ap-03, suggesting that considerable variability exists in *A. poonensis.* Disease progress was influenced by different weather factors viz., temperature, relative humidity, sunshine and evaporation. The maximum AUDPC value (area under disease progressive curve) was 322 on plants inoculated on 21st November and the lowest (171.8) was on than inoculated on 20th December in 21- 27 February, standard week followed by maximum AUDPC value 273.7 and minimum 91.0 in 14- 20 February, standard week.

Keywords: Epidemiology, Abiotic, Raunak-31, Temperature, RH, Rainfall, *Alternaria poonensis*

INTRODUCTION

Coriander (*Coriandrum sativum* L.), a major spice crop of the family Umbelliferae (or Apiaceae) is native of the Mediterranean and near Eastern Asian regions. (Saskatchwan Herb & Spice Association, 2007). Alternaria blight is one of the most devastating diseases of coriander occurring in major coriander growing states of India and World.

In India, the total area under coriander cultivation is about 321.6 lac hectares with annual productions of 2.8-3.0 lakh metric tones. In Rajasthan, coriander is cultivated in Kota, Jhalawar, Baran, Bundi, Chittorgargh and Udaipur districts on 2.81 lac hectares with production of 1.21 lac tones. (Rajasthan Agriculture Statistics, 2009-2010). Coriander crop suffers from various diseases caused by fungi and other microorganisms. Important diseases incited by fungi are stem galls/tumours (*Protomyces macrospores;* Unger, 1834), stem rot (*Sclerotinia sclerotiorum*; Korf & Dumont, 1972), wilt (*Fusarium oxysporum* f.sp. c*oriandri*; Narula & Joshi,1963), powdery mildew (*Erysiphe polygoni*; Weiltzein,1963), root and stem rot (*Rhizoctonia solani* and *Macrophomina phaseolina*; J.G.Kuhn,1858 & Goid,1947) and blight (*Alternaria poonensis;* Raghunath,1962*).* Alternaria blight of coriander is emerging as a major and wide spread problem in Rajasthan. The pathogen seems to have adaptability to higher temperatures and the disease occurs during February-April, and it is particularly severe at flowering and post flowering stages causing considerably losses to the yield.

With these facts in view, the present study on epidemiology of alternaria blight of Coriander caused by *Alternaria poonensis"* was carried out.

MATERIAL AND METHOD

The present investigation was aimed to studying the effect of environmental factors in relation to the disease development conducted at Cage House, Rajasthan College Agriculture, Udaipur, Rajasthan during *Rabi*, 2012 and 2013.

Coriander susceptible cultivar Raunak-31 was selected for this study from the grown in Cage House and was constantly examined for disease progress/decline.

Collection, isolation and purification of the pathogen

To study the populations of *Alternaria poonensis* prevalent in different places of Rajasthan, infected disease leaf samples of coriander were collected from the diseased coriander plants from the farmers fields of Kota, Lakadwas, Udaipur and Baran, RCA, Udaipur and Bhuwana, Udaipur. Cultures of coriander blight pathogen were isolated from the infected leaves. Small pieces of infected portion from diseased leaves were taken from the margin of the spots and were surface sterilized with 0.1 per cent mercuric chloride for 1 to 2 min. and were then washed in two changes of sterile distilled water for removing the disinfectant and aseptically transferred to potato dextrose agar (PDA) in Petri plates. The plates were incubated at 25 ± 2°C for growth. Sub-culture was made by removing 5 mm bit of the culture from the periphery of the mycelial growth of 4-5 days old colonies on (PDA) slants. The cultures were incubated at 25 ± 2°C for growth and sporulation. The microscopic examination of cultures indicated that the fungus belonged to the genus *Alternaria*.

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Pure culture of the pathogen was prepared by demarcating single hyphal tips on 2 per cent water agar with dummy objective which were then transferred to PDA slants and were allowed to grow. The culture was maintained at 4^0C and also by periodical transfers on PDA slants for further use.

Table 1. Isolates of *A. poonensis* recovered from samples collected from fields in coriander growing areas of Rajasthan

S.No.	Place of Collection	Cultivar	Isolated designation
1.	Kota	Local landrace	Ap-01
2.	Lakadwas, Udaipur	Local landrace	Ap-02
3.	Baran	Local landrace	Ap-03
4.	RCA, Udaipur	Local landrace	Ap-04
5.	Bhuvana, Udaipur	Local landrace	Ap-05

Pathogenicity test

Pathogenicity of the 5 isolates of *A.poonensis*, was tested by spray inoculating 21-days-old plants on pot grown plants of coriander (Raunak- 31 and Pratap raj dhaniya). The plants were raised in soil: FYM(3:1) mixture and surface sterilized seeds (0.1% $HgCl_2$) for two minutes) were sown @ 5 per pot. For preparation of the inoculum the pure cultures of different isolates were grown on PDA for 10 days on 28 ± 1^0C in Petri plates so as to allow profuse sporulation. The spores were harvested by flooding the plates with sterile distilled water and gently scrapping the colony with the help of a sterilized plastic loop and the conidial suspension was strained through muslin cloth. Final concentration of the spores was maintained 1×10^3 conidia ml^{-1}. 21-days - old plants were spray inoculated with the suspension using a hand held atomizer. The inoculated plants were kept in humid chamber for 24 hours and then transferred to cage house and high humidity was maintained throughout the disease development period by frequent irrigations.

Infection started as minute necrotic areas on all the above ground plant parts which turned purple at advanced stages and later turned brown to black during 36-71 hours and the affected parts of the plant got blighted. These typical leaf blight symptoms appeared in 7-10 days after inoculation. Re-isolation was done from infected plant parts collected 10 days after inoculation. The resultant cultures were compared with the original ones to confirm the pathogenicity.

Identification of the culture

Cultural characters of all isolates of *Alternaria*spp. were studied by growing them on PDA medium for the identification of the species and other characteristic of the fungus at 25 ± 2^0C for 10 days. Temporary mount on the slides in lactophenol and cotton blue were prepared from 10-days-old culture. These slides were examined thoroughly under the microscope for observing the characters of hyphal, colour, shape, size and septation of conidia and various cultural characters on PDA. The cultures were identified by following the standard references of *A. poonensis* (Raghunath, 1962 and Rao, 1963)

Effect of weather condition on development of alternaria blight

Staggered sowing was done from 1st Nov. and dates were as followed 7th Nov., 15th Nov., 22nd Nov. and 29th Nov.12). After germination 15-days-old plants were inoculated on 21st Nov.12, 28th Nov.12, 5th Dec.12, 12th Dec.12 and 19th Dec.2012 with a spore suspension of 1×10^3 conidia ml^{-1}. Disease severity from initiation and at interval of seven days was recorded following the 0-5 scale. Weather variables *viz*., temperature, RH, sunshine hours, rainfall and evaporation etc. were also recorded for crop season and correlation worked out.

Per cent disease intensity (PDI) was calculated based on each reading till maturity of crop. Weekly meteorological data on maximum and minimum temperature morning and evening relative humidity, rainfall and duration of sunshine hours and evaporation were obtained from agromet observatory, Agronomy farm RCA, Udaipur for the period between disease recordings to establish their correlation with disease development. Area under disease progressive curve (AUDPC) values were calculated for different recording by the formula given by Campbell and Madden (1990). Sequential apparent infection rate was calculated between each two subsequent observations. AUDPC and r_c was calculated by the method described by Vander Plank (1963), Johnson and Wilcoxson (1982) and later described by Campbell and Madden (1990) as follows:

$$AUDPC=\left[\left(\frac{X_{i+1}+X_i}{2}\right)\times(t_{i+1}-t_i)\right]$$

Where

Xi = the cumulative disease index expressed as aproportion at the i^{th} observation

ti = Time (days after planting) at the i^{th} observations.

n = Total number of observations

$$r_C=\frac{1}{t_2-t_1}\log e\frac{n_2(1-n_1)}{n_1(1-n_2)}$$

Where t_1 = Time (days) during the 1st observations

t_2 = Time during the 2nd observation.

$t_2 - t_1$ = Time interval between two observations and subsequently so on.

n_1 = Per cent disease index value in decimal at corresponding t1 time.

n_2 = Per cent disease index value at t2 time.

The disease severity was recorded on standard 1 to 5 disease rating scale. Details of the disease rating scale are given below:

Per cent all above ground parts infected	Score
Free from disease	0
1to10% area of leaf and umbel blighted	1
11 to 20% area of leaf, stem and umbel blighted	2
21 to 35% area of leaf, stem and umbel blighted	3
36 to 60% area of leaf, stem and umbel blighted	4
More than 61% area of leaf, stem and umbel blighted	5

The per cent infection index (Mckinney, 1923; Chester, 1959 and Wheeler, 1969) was calculated as

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of all individual disease rating}}{\text{Total No. of plants ass. x maximum rating}} \times 100$$

RESULT AND DISCUSSION

The experiment was conducted to find out the effect of environmental factors on the development of Alternaria blight on the susceptible coriander cultivar Raunak-31 of coriander in *Rabi* 2012-13. Weekly sowing was done start from 1st Nov. and the following dates were 7th Nov., 15th Nov., 22nd Nov. and 29th Nov.). After germination 15-days-old plants were inoculated with virulent isolate Ap-01 with 1×10^{-3} ml^{-1} spore suspension, to know the relationship between the disease severity (dependent variable) and the weather factors (maximum temperature, minimum temperature, maximum per cent relative humidity and minimum per cent relative humidity) multiple linear regression analysis and for (sunshine, rainfall and evaporation), simple regression analysis was done with 1st Nov. sown plants. By fitting this equation, the contribution of weather factors in the development of Alternaria leaf spot was observed.

Table 2.

S.No.	Disease severity	Independent value				
	PDI on 1st sown	Weather factors	R	R^2	A	B
1.	4.4	T max.	-0.225	0.051	64.105	-1.733
2.	9.3	T min.	-0.017	0.00	19.999	-0.092
3.	25.4	RH max.	0.110	0.012	-20.813	0.506
4.	36.6	RH min.	0.246	0.060	-5.174	0.909
5.	41.1	SSH	0.425	0.181	-60.906	9.736
6.	50.4	Evap.	0.287	0.083	4.399	4.902

$Y = a + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_4 X_4$

R^2 = 0.8972

a = -321.714

b_1 = 3.602

b_2 = -2.414

b_3 = 2.0142

b_4 = -0.6834

Thus, the equation comes to:

$Y = -321.714 - 3.609\ X_1 - -2.414\ X_2 + 2.0142\ X_3 - -0.6834\ X_4$

b_1 = 3.602: It means that holding X_2 (minimum temperature), X_3 (maximum relative humidity) and X_4 (minimum relative humidity), constant a 1 per cent increase in X_1 (maximum temperature), led on average to about 1.694 per cent decrease in Y (per cent disease index)

b_2 = -2.414: It means that holding $X_1 = X_3$ and X_4 constant a 1 per cent increase in X_2 lead on average to about -7.715 per cent increase in Y.

R^2 = 89 per cent, it means variation at 89 per cent explained by dependent variable (disease severity) and remaining 11 per cent variable is unexplained.

r = 0.370 (Sunshine):

The correlation coefficient between average per cent disease index and sunshine hrs was 0.370, it indicates is a positive correlation, between the in per cent disease index, indicating higher disease severity with the increase in sunshine.

r = - 0.246 (Evaporation):

There is negative correlation between average per cent disease index and evaporation (-0.246), it indicate that increase in evaporation resulted in decrease in per cent disease index.

Table 3. Comparative pathogenic potential of different isolates of *A. poonensis* on pot grown plants of coriander cultivar Raunak-31

S.No.	Isolate	Latent period (in hrs)	Percent disease index
1.	Ap-01	38	53.2 (46.84)
2.	Ap-02	52	49.8 (44.89)
3.	Ap-03	73	46.3 (42.88)
	SEm±		0.92
	CD at 5%		2.80
	CD at 1%		3.88

Table 4. Progression of Alternaria blight on coriander in relation to weather parameters with different date of date of sowing during *Rabi* 2012-13

Standard week	Metrological weeks	Temperature (°C)		Relative humidity (%)		Sunshine (hrs)	AUDPC*				
							Inoculated on				
		Max.	Min.	Max.	Min.		21st Nov.	27th Nov.	5th Dec.	13th Dec.	20th Dec.
47	21Nov.-27Nov.12	30.1	12.6	80	45.7	8.5	15.4	0	0	0	0
48	28Nov.-4Dec.	27.2	12.1	84.9	41.3	6.8	35.7	9.8	0	0	0
49	5Dec.-12Dec.	30.4	13.5	81.4	43.6	8.9	45.1	22.4	7.0	0	0
50	13Dec.-19Dec.	25.4	6.4	76.3	33	8.6	53.9	25.9	14.3	5.6	0
51	20Dec.-26Dec.	26.5	7.6	87	41.1	7.7	60.9	27.3	16.4	11.5	5.6
52	27Dec.-2Jan.13	24.2	7.4	95.5	41	7.2	64.4	28.7	18.5	11.9	11.2
1	3Jan.-9Jan.	23.0	4.4	85.6	30.7	7.8	65.4	30.4	18.9	13.3	12.2
2	10Jan.-16Jan.	23.1	6.4	75.3	21.1	8.5	71.7	38.1	26.6	22.0	16.1
3	17Jan.-23Jan.	23.9	7.5	80.3	28.1	7.6	97.6	68.2	51.4	44.8	25.5
4	23Jan.-30Jan.	22.7	4.0	74.9	19.6	9.0	147.7	124.9	76.3	64.4	35.7
5	31Feb.-6Feb.	25.9	10.0	76.3	26.9	5.7	209.3	189.7	105.0	77.7	39.9
6	7Feb.-13Feb.	24.3	7.6	78	28.2	8.3	248.5	216.1	130.2	100.8	52.5
7	14Feb.-20Feb.	26.3	11.7	83.3	34.3	8.3	273.7	235.7	175.0	159.6	91.0
8	21Feb.-27Feb.	26.8	10.6	82	31	8.7	322	288.4	255.1	250.6	171.8

*Mean of five replications*Observation started 7 days after inoculation and at weekly intervals (please sees Materials & Methods)

1st sown-1st Nov., 2nd -7th Nov., 3rd – 15th Nov., 4th -22nd Nov. and 5th -29th Nov.12

Considerable variations were observed in AUDPC in five different dates of sowing and correlation with weather factors on disease development. In the first planting on 21st Nov. the AUDPC ranged 15.4, in the next week it was 35.4 and in the third week (42 days old plant) it was 45.1. The AUDPC in the following 7 weeks ranged for 60.9-147.7. In the 8th week the AUDPC given high (209.3-322) in the plants sown on 28th Nov.2012.

In the plants sown on 7th Nov. and inoculated on 28th Nov. the AUDPC in the first week after inoculation (28th Nov.- 4th Dec.) was 9.8. It progressed slowly and up to next six weeks (up to 9th Jan.) AUDPC ranged between 22.4- 30.4. In the following weeks it increased slightly and was 38.1, but after that there was a sudden increase in AUDPC to almost double, then it was 124.9, 189.7, 216.1, 235.7 and 288.4.

In the plants sown on 15th Nov. and inoculated on 5th Dec., the AUDPC was at first lower (7.0) than the first two dates of sowings (15.4 & 9.8 respectively) and from 2nd week to 5th week (16th Jan. 2013), it remained in the range of 14.3-26.6. But in the next week there was sudden increase and AUDPC reached to 51.4, 76.3, 105.0, 130.2, 175 and 255.1.It was lower than that observed there stages in the first two sowings. Similarly, in the plants sown on 22nd Nov. and inoculated on 5th Dec. and also than sown on 29th Nov. and inoculated on 19th Dec. the AUDPC was 5.6. It increased to 11.5 & 11.2, respectively on the next week and remained in the range of 11.9-22.0 during next four weeks. But there were difference in

the two dates of sowing after 4th weeks. Higher AUDPC was recorded in the 5th week in plants sown on 22nd Nov. (44.6) as compared to 25.5 in those sown on 29th Nov. In the former set, the AUDPC in the following weeks was 64.4, 77.7, 100.8, 159.6 & 250.6, while on the later sown; the AUDPC on the 5th week much was lower and was 35.7, 39.9, 52.5, 91.0 and 171.8 only.

It was observed that the disease progressed faster when the maximum temperature ranged from 24.3 to 26.8^{0}C and minimum temperature was from 7.6 to 11.7^{0}C as compared to 22. To 24.2^{0}C maximum and 4.0 to 10.0^{0}C minimum during 27th Dec.2012 to 3 Jan.2013.The age of the plant also seemed to be important as disease progress was higher on 12-15 week old plants.

Studies on the effect of weather factors on disease development revealed that 21 Nov. to 10 Jan. 2013 due to 30.7-95.5 per cent relative humidity and 13.5-30.4^{0}C temperature range AUDPC was 71.1 on 21st Nov. sown plants followed by 38.1, 26.6, 22.0 and 16.1 on 27th Nov., 5th Dec., 13 Nov. and 20th Nov. sown plants. During 11 Jan. to 24 Jan.2013 was moderate period for Alternaria blight development as during this period the minimum and maximum relative humidity ranged between 21-1-30.7 and per cent coupled with minimum and maximum temperature i.e., 4.4-7-5^{0}C and 23-25.1^{0}C and AUDPC was 147.7 on 21st sown plants followed by 124.9, 76.3, 64.4 and 35.7 on 27th Nov., 5th Nov., 13 Nov. and 20th Nov. sown plants.

The period between 25 Jan. to 27 Feb. 2013 was much favourable for disease development and more severity during this period was observed in comparison to progressive phase. The minimum and maximum temperature range was 4-11.7^{o}C and 22.7-26.8^{o}C accompanied with minimum and maximum relative humidity i.e., between 19.6-31 and 74.9-83.3 per cent respectively in progress phase AUDPC value ranged from 250-322 per cent while disease was found almost stable. The area under disease progressive curve (AUDPC) is a quantitative measure of disease intensity with time. It is used in plant pathology to indicate and compose levels of resistance to disease among different varieties of crops, effect of weather factors and various disease suppression treatments. It is preferred by using a formula devised by Campbell and Modden (1990). Lower AUDPC represented slower disease progression and the high AUDPC represents faster disease progression. In the present study, AUDPC values were lower during 21st Nov. to 27th Dec. when temperature ranges in 7.6-30.4^{0}C and relative humidity 33-87 per cent. The AUDPC value was moderate (ranging from) during 28th Dec.12 to 7th Feb.13. With increase in Temperature (7.6-26.8^{0}C) during 8th Feb. to 27th Feb.13 period, the disease progression was much faster and high AUDPC values were obtained. It appeared that despite different ages of the coriander, the temperature of 7.6 to 26.8^{0}C were the most confined during 8th Feb. to 27th Feb.13.

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RESEARCH ARTICLE

QUANTITATIVE ESTIMATION OF SEED PROTEIN AND ESSENTIAL OIL CONTENT IN EIGHT PLANT TYPES OF FENNEL (*FOENICULUM VULGARE* MILL.)

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Abstract: Investigation highlights quantitative estimation of seed protein and essential oil contents (from M_3 harvested seeds) in seven macromutants (screened at M_2), along with control. Results indicate that in comparison to control protein content enhance in *early flowering* mutant; while, essential oil content is higher in *thick stem*, *slender stem*, *pigmented stem* and *elongated pinnae* mutants. It opens up the scope of efficient breeding for raising desirable 'plant types' of interest.

Keywords: Fennel, Macromutants, Estimation, Seed, Protein

INTRODUCTION

Fennel (*Foeniculum vulgare* Mill.; Family-Umbelliferae) is a spice yielding plant of commerce and is cultivated worldwide (Muckensturm *et al.*, 1997; Grover *et al.*, 2013). Apart from spice yielding property, fennel possess immense therapeutic (Özbek *et al.*, 2003; Choi and Hwang, 2004; Tognolini *et al.*, 2007; Pradhan *et al.*, 2008; Mohamad *et al.*, 2011; El-Soud *et al.*, 2011; Saini *et al.*, 2014) and nutritional (Barros *et al.*, 2010; Blazewicz-Wozniak, 2010; Das *et al.*, 2013; Badgujar *et al.*, 2014) values. Therapeutic potentiality is mainly due to presence of essential oil in seeds and foliage (Blazewicz-Wozniak, 2010; Taie *et al.*, 2013). Sustainable work on clinical aspects of fennel has been performed (Oktay *et al.*, 2003; Joshi and Parle, 2006; Mohsenzadeh, 2007; Faudale *et al.*, 2008; Shahat *et al.*, 2011; Koppula and Kumar, 2013) but limited efforts (Ramkrishna, 2008; Mostafa and Abou Alhamd, 2015) have been focused on genetic manipulation of the species. With the view to it, the authors have initiated an induced mutagenesis programme to create desirable mutants rich in essential oil as well as nutritional content. Present investigation reports on the quantitative estimation of seed protein and essential oil content in control and mutant plant types of fennel.

MATERIAL AND METHOD

In an induced mutagenesis (EMS and γ-irradiations) programme, seven macromutants (*thick stem*, *slender stem*, *pigmented stem*, *dwarf*, *elongated pinnae*, *narrow pinnae* and *early flowering*) were screened in M_2 generations; the mutant traits were confirmed at M_3. Protein and essential oil content was estimated from control and all macromutants (three replicas in each case) of fennel using selfed M_3 harvested seeds. Extraction of soluble protein from seed was done following Osborne (1962) and estimated quantitatively as per the method of Lowry *et al.* (1951). Quantitative estimation of essential oil was made also from seeds in controls and in macromutants (M_3 harvested seeds) following hydrodistillation process as was suggested by Simon *et al.* (1990). Two grams of dry seeds were used in each set of experiment (one set=one replica; three replicas for each plant type). The seeds were crushed slightly (to break the mericarp) before use and 2 to 3 hours extraction time has been given for each sample. The oil extracted (room temperature) was separated from water (with diethyl ether) in a separating funnel and measured in a microgradiated tube designed for the purpose (data obtained for each plant type were pooled).
It is essential to note that seeds used were of identical maturity and sun dried for 2 consecutive days, 4 hour each day.

RESULT AND DISCUSSION

Protein and essential oil content of control fennel seed are 11.94% and 7.0% respectively (Table 1). In macromutants protein content varies from 7.28% (*thick stem* mutant) to 16.78% (*early flowering* mutant) and essential oil content range from 6.0% (*late flowering* mutant) to 8.0% (both in *thick stem* and *slender stem* mutant). Protein content enhances in *early flowering* mutant only than control. Blazewicz-Wozniak (2010) showed that protein content is related to sowing time.
Essential oil content is higher in *thick stem*, *slender stem*, *pigmented stem* and *elongated pinnae* mutants

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than control. Previous reports suggest that essential oil content depends on fruit maturity (Telci *et al.*, 2009), application of fertilizer (Khan *et al.*, 1992; 1999; Ali *et al.*, 2012), spraying of salicylic acid (Hashmi *et al.*, 2012), method of hydrodistillation (Mimica-Dukic *et al.*, 2003) among others. In the present study no fertilizer is applied during any stage of cultivation. From the result it is evident that none of the mutants is superior to control both in protein and essential oil content but few of them show betterment in either parameter.

Table 1. Seed protein and essential oil content in 8 plant types (control and macromutants).

Plant types	Protein content (%)	Essential oil content (%)
Control	11.94	7.0
thick stem	7.28	8.0
slender stem	9.28	8.0
pigmented stem	8.60	7.3
dwarf	10.60	7.0
elongated pinnae	11.33	7.7
narrow pinnae	11.23	7.0
early flowering	16.78	6.0

CONCLUSION

This result opens up the possibility of direct selection of these superior plant types as well as offer scope of further improvement through hybridization followed by proper selection.

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RESEARCH ARTICLE

EFFECT OF TEMPERATURE, PH AND VARIOUS MEDIA ON GROWTH AND SPORULATION OF *TRICHODERMA* SPP. ISOLATES FROM UTTAR PRADESH

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Abstract: *Trichoderma* spp. isolates were collected from different chickpea fields of Sultanpur, Sitapur, Barabanki, Kanpur Nagar and Etawah (Uttar Pradesh). These isolates were tested to study growth and sporulation behavior of *Trichoderma* sp. at different Temperature, pH and media. The most favorable temperature for growth and sporulation of *Trichoderma* sp. was found 30^0C (74.33mg), followed by 25^0C where average growth of the bio-agent was recorded as 64.66mg. Similarly the most favorable pH ranges was found 6.5 - 7.5 in which total dry weight of mycelium also varies between 200.33 to 226.33 mg and also very good sporulation was observed. The minimum dry weight was recorded as 109.66 at pH 3.0. Among the different media (*viz*., Potato dextrose Agar, Rose Bengal Agar, Asthana and Hawker's Agar, Sabouraud's Agar and Czapek's (Dox) Agar) Potato Dextrose Agar (PDA) shows excellent in average colony diameter (8.09 cm) followed by Rose Bengal Agar (7.69 cm), but excellent average mycelium weight (176.66 mg) was recorded in Potato Dextrose Broth (PDB) medium and also excellent sporulation were observed on Potato Dextrose and Rose Bengal broth. Studies on the biology of *Trichoderma* sp. isolates at different temperature and pH conditions is helpful for practical utility to contain disease problems in agro-ecosystems.

Keywords: Effect, Temperature, Trichoderma, Uttar Pradesh

INTRODUCTION

Trichoderma spp. are free-living fungi that are highly interactive in root, soil and foliar environments. *Trichoderma* species are widespread saprophytic soil borne or wood-decaying fungi, which appear to be well adapted to diverse abiotic stresses such as salinity and drought (Kubicek *et al*., 2002). It has been known for many years that they produce a wide range of antibiotic substances and that they parasitize other fungi. They can also compete with other microorganisms; for example, they compete for key exudates from seeds that stimulate the germination of propagules of plant-pathogenic fungi in soil and, more generally, compete with soil microorganisms for nutrients and/ or space. These direct effects on other fungi are complex and remarkable and, until recently, were considered to be the bases for how *Trichoderma* spp. Exert beneficial effect on plant growth and development and were exploited commercially for management of diverse soil borne pathogens (Papavizas and Lumsden 1982, Samuels 1996). However, in addition to the ability of *Trichoderma* spp. to attack or inhibit the growth of plant pathogens directly, recent discoveries indicate that they can also induce systemic and localized resistance to a variety of plant pathogens. Moreover, certain strains also have substantial influence on plant growth and development. Study on growth and sporulation would dramatically change our knowledge and gives effective strategy for recommendation in new areas and crucially important for agricultural uses and for understanding the roles of *Trichoderma* in natural and managed ecosystems. The study was carried out to know the effect of different Temperature, pH and media on growth and sporulation of *Trichoderma* sp.

MATERIAL AND METHOD

Soil samples were collected from chickpea fields of Sultanpur, Sitapur, Barabanki, Kanpur Nagar and Etawah (UP) and listed in table (1). All the culture media were sterilized in autoclave at 1.1kg/cm^2 for 20 minutes at 121.6^0C. The glasswares cleaned in chromic acid (potassium dichromate 60 gm, sulphuric acid 60 ml and distilled water 100 ml) and properly washed 2-3 times by distilled water. Bio-agents were then isolated and purified by serial dilution methods and were preserved in PDA. These cultures were then deposited at ITCC, Division of Plant Pathology, IARI for proper identification and accessioned as ITCC 7442,7443,7444,7450 and 7451 for five cultures.

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Table 1. List of used isolates

I.D. No	Culture type	Ref. No.	Source	Fungus identified
ITCC-7442/09	6 CP	01	Sultanpur	*Trichoderma atroviride*
ITCC-7443/09	24 CP	02	Sitapur	*Trichoderma atroviride*
ITCC-7444/09	28 CP	03	Barabanki	*Trichoderma longibrachiatum*
ITCC-7450/09	5 CP	04	Kanpur Nagar	*Trichoderma longibrachiatum*
ITCC-7451/09	105 CP	05	Etawah	*Trichoderma atroviride*

Growth on media

The studies were carried out to determine the linear hyphal growth by growing in five solid media including Potato dextrose Agar, Rose Bengal Agar, Asthana and Hawker's Agar, Sabouraud's Agar and Czapek's (Dox) Agar in three replications. On liquid media *viz*., Potato dextrose, Rose Bengal, Asthana and Hawker's, Sabouraud's and Czapek'sDox the amount of mycelial mat was determined.

Effect of different temperatures on growth of Bio-Agents

To find out optimum as well as suitable temperature for the growth of *Trichoderma* sp., the microorganism was grown at 5 different temperatures on potato dextrose agar medium. After 10 days of incubation the average mycelial dry weight was recorded

Effect of pH on growth and sporulation of Bio-agents

pH value plays an important role for growth and spore germination. The set of different pH values (3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) was prepared and pH was adjusted by adding appropriate amount of citrate phosphate buffer. For each pH value, there were three replications. The spore count was recorded by using haemocytometer as $1x10^5$ spore/ ml.

RESULTS AND DISCUSSION

In the present study *Trichoderma* spp. isolates were collected from chickpea fields Sultanpur, Sitapur, Barabanki, Kanpur Nagar and Etawah (UP). These isolates were tested to know growth and sporulation behavior at different Temperature, pH and media. The highest radial growth of the bio-agent was recorded on Potato Dextrose Agar medium (8.09 cm) followed by Rose Bengal Agar medium (7.69 cm) (Table: 2). Radial growth of the bio-agent on other media like Asthana and Hawker's (7.62) and Sabouraud's (6.96) have shown good growth and was at par with the PDA. Growth on Czapek'sDox medium was found average (1.69). The highest mycelial weight was recorded in Potato Dextrose broth (176.66 mg) followed by Rose Bengal broth (165 mg), Asthana& Hawker's (129.33mg), Sabouraud's (87.66 mg) and Czapek's (Dox) (28.66 mg) (Table:3).

Table 2. Effect of solid media on growth and sporulation

Solid Media	Average colony diameter in (cm)	Category of fungal growth	Nature of colony and sporulation
Potato Dextrose Agar	8.09	Excellent	Heavy sporulation, greenish colour
Rose Bengal Agar	7.69	Excellent	White to gray colour colony, compact grayish green, profuse sporulation
Asthana and Hawker's	7.62	Good	White to light gray colour colony and heavy sporulation

Sabouraud's Agar	6.96	Good	Light gray, compact and heavy sporulation
Czapeks (Dox) Agar	1.69	Average	White thin, flaky growth restricted
CD (0.05)	0.012		

Table 3. Effect of liquid media on growth and sporulation

Liquid Media	Average mycelium wt. in (mg)	Category of sporulation
Potato Dextrose Broth	176.66	Excellent
Rose Bengal Broth	165.00	Excellent
Asthana& Hawker's	129.33	Good
Sabouraud's	87.66	Fair
Czapek's (Dox)	28.66	Poor
CD (0.05)	3.08	

Four types of sporulation on media were observed, the excellent sporulation of the bio-agents were recorded on Potato Dextrose and Rose Bengal broth media. Good sporulation was observed on Asthana and Hawker's medium. However, fair sporulation was observed in Sabouraud's and Czapek'sDox media. These results are in complete conformity with previous studies on other organisms. Ashour and El-Kadi, (1959) and Rangaswami and Sambandam, (1960) also found potato dextrose agar as the best medium for the linear growth of the fungus *Alternaria alternata.* The potato dextrose broth was found the best liquid media for growth and sporulation of *Alternaria helianthi* (Gupta *et al*., 1969), *Alternaria pori* (Raju and Mehta, 1982) and *Colletotrichum dematium* var. *truncate* (Kumar and Dubey, 2007).

In assessing the optimum as well as suitable temperature for the growth of *Trichoderma* sp. the microorganism was grown at 5 different temperatures on potato dextrose agar medium. The growth of the pathogen increased up to 30^0C temperature, thereafter the growth starts decreasing (Table: 4). Interestingly maximum average dry weight (74.33 mg) of the mycelium was observed at 30^0C temperature. The statistical analysis shows that the growth of the bio-agent significantly affected with either increase or decrease of the temperature. Similarly, some of the researchers also reported that 25-30^0C was suitable for growth and sporulation of *Trichoderma* (Kunming, 2004; Singh and Sudhir, 2009; Shahid *et. al*. 2011). Moreover, Sharma *et. al.,* (2005) reported that none fungi could grow at temperature 40^0C.

Table 4. Effect of temperature on growth of *Trichoderma* spp.

Temperature (ºC)	Average dry weight of the bio-agent (mg)
15	21.41
20	42.30
25	64.66
30	74.33
35	41.66
CD at 5% (0.05)	0.82

Significantly highest weight was recorded as 226.33 mg at pH 7 followed by 200.33 mg at pH 6.5 (Table: 5). The minimum dry weight was recorded as 109.66 at pH 3.0. Thus, excellent sporulation was recorded at pH 6.5 – 7.0. It has also been observed that neither alkaline nor acidic conditions are congenial for the growth and sporulation of the bio-agent. The results are in conformity with the findings of Kunming (2004), who reported that *Trichoderma harzianum* strains (Th-B) grew better at pH 5-8. The pH range 7 – 7.5 has significant influence on the growth and sporulation on *T. atroviride* (Singh *et. al*., 2011).

Table 5. Effect of pH on growth and sporulation of *Trichoderma* spp.

Range of pH	Total wt. (mg)	Sporulation
3.0	109.66	Poor
4.0	117.33	Poor

4.5	138.33	Fair
5.0	163.33	Fair
5.5	196.66	Good
6.5	200.33	Excellent
7.0	226.33	Excellent
7.5	179.33	Good
8.0	122.66	Good
CD at 5% level	3.02	Poor

CONCLUSION

In the present study temperatures, pH and media (solid and liquid) had a significant influence on the growth and sporulation on *Trichoderma* sp. Among the different media, PDA shows excellent in average colony diameter (8.09 cm) followed by Rose Bengal Agar (7.69 cm), but excellent average mycelium weight (176.66 mg) was recorded in PDB medium. The most favorable temperature for growth and sporulation of *Trichoderma* sp. was found 30^0C (74.33mg), followed by 25^0C where average growth of the bio-agent was recorded as 64.66mg. Similarly, most favorable pH ranges was found 6.5 - 7.5 in which total dry weight of mycelium also varies between 200.33 to 226.33 mg. Variability of five isolates of *Trichoderma* sp. collected from rhizosphere soil of different places of Uttar Pradesh have been revealed that variability exists among the isolates. These findings can be utilized for recommendations of these bio-control agents for different agro ecological regions.

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RESEARCH ARTICLE

EFFICACY OF MEDICINAL PLANT LEAF EXTRACTS, OILS AND BIOAGENTS AGAINST *RHIZOCTONIA SOLANI* CAUSING AERIAL BLIGHT OF SOYBEAN

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Abstract: Soybean (*Glycine max* (L.) Merrill) is one of the most important oil seed crop of India .It was wonder of the twentinth century. Soybean ranks first among world oilseed with an annual production of about 105 mt. In Chhattisgarh, the crop is grown over an area of 0.82 m ha with production and productivity of 0.73 mt and 891 kg/ha, respectively which are much lower than national average. Soybean aerial blight caused by *Rhizoctonia solani* is a most important oilseed disease. The disease appears during July-August and is characterized by sudden and complete death of the plants. This disease is very destructive and causes heavy losses to the tune of 35-60 % in warm and humid parts of the countries. Antifungal activity of different medicinal plant leaf extracts, oils and *Trichoderma spp* were studied under *in vitro* condition. Out of fifteen medicinal plants studied, the leaf extracts of Butch significantly inhibited the mycelial growth of *Rhizoctonia solani* under *in vitro* conditions. Among the medicinal oils, Eucalyptus and Neem oils were found to significantly inhibit the mycelial growth of *Rhizoctonia solani* at 5% concentrations. Among the antagonists, maximum mycelial growth inhibition was caused by *Trichoderma harzianum* (74.81%) followed by *Trichoderma viride* (67.40%) while *Trichoderma spp.* (mushroom isolates) was least effective against *Rhizoctonia solani*.

Keywords: Soybean, *Rhizoctonia solani*, Antifungal compound, *Trichoderma spp.*

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is one of the most important oil seed crops of India .It was wonder of the twentieth century. Soybean ranks first among world oilseeds with an annual production of about 105 mt. Among the different growing countries of the world, USA, China, Brazil, Argentina and India are major producers which accounts for more than 90% of the world's acreage (Taware *et al.*, 2007). Soybean is mainly grown during Kharif season in sandy loam to clay loam soil in Chhattisgarh. In Chhattisgarh, th crop is grown over an area of 0.82 m ha with production and productivity of 0.73 mt and 891 kg/ha, respectively which are much lower than national average. (Anonymous, 2006). Soybean aerial blight is a most important oilseed disease. The disease appears during July-August and is characterized by sudden and complete death of the plants. This disease is very destructive and causes heavy losses to the tune of 35-60 % in warm and humid parts of the countries (Patel *et al.*, 1998). Although various fungicides have shown promising results in controlling the aerial blight of soybean but the phytotoxicity and fungicidal residue problems leading to the environmental pollution are the major constraints in disease management. Substantial emphasis is being given these days on using eco-friendly approaches for controlling plant diseases. Several medicinal plants especially neem, eucalyptus and butch were reported to be one of the best alternatives to synthetic fungicides. In same context, an attempt was made through this investigation, to evaluation of different antifungal compounds against *Rhizoctonia solani* causing aerial blight of soybean.

MATERIAL AND METHOD

Leaf extracts of medicinal plants

Antifungal activity of fifteen medicinal plant leaf extracts was studied under *invitro*. The medicinal plants viz., Lemon grass (*Cymbopogan flaxuosus*), Bhringraj (*Wadelia chinensis*), Kalmegh (*Andrographis paniculata*), Ashwagandha (*Withania somnifera*), Satawar (*Asparagus racemosus*), Butch (*Acorus calamus*), Mandukparni (*Centella asiatica*), Bramhi (*Bacopa moniari*), Patchouli (*Pogostemon patchouli*), Vantulsi (*Hyptis suaveolens*), Eucalyptus (*Eucalyptus globulus*), Besrum (*Ipomea spp*), Neem (*Azadirachta indica*), Karanj (*Pongamia pinnata*) and Datura (*Datura stramonium*) were used. PDA without extract was used as control. The procedure for preparation of leaf extract medium was same as for standard PDA medium. Twenty gm leaves of each medicinal plant were taken in 100ml water and boiled till they were softened. Softened medicinal plant leaves were cursed in pastel and mortar, and then extract was filtered. Two gm of dextrose and two gm agar- agar were mixed in filtered leaf extracts and volume was made up to 100 ml followed by autoclaving at 15 lbs pressure for 20 minutes. In each sterilized petriplates 20 ml media was poured and allowed to solidify. A 5 mm disc from 4 days old culture of test fungus was placed in the centre of medium. Three replications were maintained for each treatment along with a control. The inoculated petriplates were incubated in the BOD incubator at 27±2 ^{o}C and observation were recorded at 3 and 5

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days after incubation. The percent growth inhibition of pathogen was calculated as below:

$$\text{Percent growth inhibition} = \frac{\text{Growth of test pathogen} - \text{Growth of test pathogen in control plate in presence of leaf extract}}{\text{Growth of test pathogen in control plates}} \times 100$$

Oils of medicinal plants

Antifungal activities of different medicinal plant oils were studied under *in vitro* condition. The medicinal oils *viz.,* Alsi (*Linum usitaticimum*), Til (*Sesamum indicum*), Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus globulus*), Arandi (*Ricinus communis*), Mahua (*Maduca indica*), Karanj (*Pongamia pinnata*) and Mustard (*Brassica compestris*) were used. PDA without oil was used as control. To evaluate the bio efficacy of medicinal oils at 5 % concentration, 5 ml of each oil was mixed in 95 ml PDA in each conical flask of 250 ml capacity. There after autoclaving was done at 15 lbs for 20 minutes. Twenty ml media was poured in each of the sterilized petriplates of 90 mm diameter and allowed to solidify. On solidification, 5 mm disc of 3 days old culture of test fungus was placed in the centre of the plates. Three replications were kept in each treatment along with control. Inoculated petriplates were incubated in the BOD incubator at 27±2 ^{o}C and observations were recorded at 1, 2 and 3 days after inoculation and the percent growth inhibition of pathogen was calculated as described above for leaf extracts..

Bioagents

The pure cultures of *Trichoderma viride* and *Trichoderma harzianum* were obtained from Department of Plant Pathology, Indira Gandhi Agricultural University, Raipur, Chhattisgarh. The culture of *Trichoderma spp*. (Mushroom isolates) were obtained from paddy straw mushroom beds. The antagonistic activity of these isolates against *R. solani* was evaluated by dual culture technique. An amount of 20 ml sterilized melted PDA was poured in 90 mm diameter petriplates. After solidification of medium, 5 mm disc of the antagonist and the test pathogen were separately cut with the help of a sharp sterilized cork borer from the edge of 3 days old culture and placed in straight line at distance of 5 mm from the edge. In control plates antagonist was replaced with the test fungus. The inoculated petriplates in triplicate were incubated at 27±2 ^{o}C. Observation was recorded on the radial growth of the antagonist and test pathogen when the fungus in control plate reached to rim of the plate. The per cent growth inhibition of the test pathogen in presence of antagonist *was* calculated over control as bellow.

$$\text{Percent growth inhibition} = \frac{\text{Growth of test pathogen} - \text{Growth of test pathogen in control plate in presence of } \textit{Trichoderma spp.}}{\text{Growth of test pathogen in control plates}} \times 100$$

RESULT AND DISCUSSION

Leaf extracts of medicinal plants

Fifteen medicinal plant leaf extracts were evaluated to study the antifungal activity on the growth of *R. solani* at 3 and 5 days after inoculation (Table 1). The per cent inhibition in mycelial growth of *R. solani* ranged from 12.83 % to 87.71 %. The maximum inhibition in mycelial growth was recorded in the extract of Butch (87.71%) followed by Eucalyptus *(*75.93 %). Both treatments were statistically at par with each other at 3 DAI. The percent mycelial growth inhibition by different plant extracts at 5 DAI ranged between 0.00 to 87.04 %. The maximum mycelial growth inhibition was recorded in plant extract of Butch (87.04 %) followed by Eucalyptus (66.66 %). These two leaf extracts were found to be significantly superior to leaf extracts of other medicinal plants (Table 1). Tiwari *et al.* (2007) also tested the efficacy of medicinal plant extracts *in vitro* against *R. solani* and reported that out of 950 extracts, *Acorus calamus* (Butch) was highly effective against *R. solani* at different concentrations (1%, 5% and 10%). Ansari (1995) also reported fungistatic activity of Eucalyptus extract against *R. solani*. Similarly Reddy *et al.* (2002) reported that extract of *Eucalyptusglobulus, Allium sativam* and *Zingiberoffinale* caused 61 to 100 % inhibition of the mycelial growth of *R. solani* causing root rot of chickpea. Sharma *et al.* (2005) tested the efficacy of eight plant extracts against *R. solani in vitro* and reported that *Eucalyptus globulus* inhibited 85% mycelial growth at 10% concentration. These results clearly suggest that butch and eucalyptus can be used as best alternatives to synthetic fungicides and can be incorporated in Integrated Disease Management (IDM) module to manage the aerial blight of soybean.

Oils of medicinal plants

Eight medicinal plant oils were evaluated for the effect on the growth of *R. solani* at 5 % concentration (Table 2). Maximum mycelial growth inhibition was recorded in Eucalyptus oil (100 %) followed by Neem (86.78, 71.85 and 49.26 %) at 1, 2 and 3 days after inoculation respectively. These two medicinal plant oils were found significantly superior to rest of the tested medicinal plant oils (Table 2). Madhukar and Reddy (1989) also reported that Eucalyptus oil completely checked the fruit rot diseases of guava caused by *R. solani* and anthracnose caused by

Pestalotiopsis versicolor. Similarly Singh *et al.* (1989) evaluated 6 oils of medicinal plants for their antifungal activity against *Sclerotium rolfsii* and 10 soil inhabiting fungi. Out of these, the oil of *Azadirachta indica* was most effective followed by *Eucalyptus globulus*. These results clearly suggest that eucalyptus and neem can be used as best alternatives to synthetic fungicides and can be incorporated in Integrated Disease Management (IDM) module to manage the aerial blight of soybean.

Bioagents

The data presented in Table 3 revealed that all the isolates of *Trichoderma* inhibited mycelial growth of *R. solani* by 55.77 to 74.81 percent over control. Minimum mycelial growth of *R. solani* was recorded in *T. harzianum* (22.67mm) followed by *T. viride* (29.34mm). It is concluded from the above data that *T. harzianum* isolates was most effective species to inhibit the mycelial growth of *R. solani*. These observations are agreement with the finding of Sharma and Shankran (1996) and Talanca (1999) who also reported the antagonistic activity of *Trichoderma spp*.. These results clearly suggest that *T.harzianum* can be used as potential biocontrol agent against *R. solani* and can be incorporated in Integrated Disease Management (IDM) module to manage the aerial blight of soybean. Ray *et al.* (2007) also tested the efficacy of bio-agents under *in vitro* condition. Among the bio-agents, *T. harzianum* found most effective as it inhibited the mycelial growth of *R. solani* after 96 hr of incubation followed by *T. viride* and *P. flourescens* where 82.43 and 80.36 mm growth were observed, respectively. Sarojaini and Nagmani, (2007) and Cundom *et al.* (2003) tested the antagonistic potential of *Trichoderma* isolates against *Rhizoctonia solani* and found that all the isolates inhibited the mycelial growth of *R. solani* in dual cultures.

Table 1. Evaluation of leaf extracts of medicinal plants against *Rhizoctonia solani* under *in-vitro* condition

S.N.	Medicinal plants	3 DAI**		5 DAI**	
		Mycelial growth (mm)*	% inhibition	Mycelial growth (mm)*	% inhibition
1	Lemongrass	35.66	42.78	57.50	36.11
2	Bhringraj	44.50	28.60	90.00	0.00
3	Kalmegh	45.50	27.00	90.00	0.00
4	Ashwagandha	28.83	53.74	53.33	40.74
5	Satawar	54.33	12.83	90.00	0.00
6	Butch	7.66	87.71	11.66	87.04
7	Mandukparni	47.50	23.79	90.00	0.00
8	Brahmi	31.16	50.00	59.16	34.26
9	Patchouli	47.66	23.53	90.00	0.00
10	Vantulsi	38.16	38.77	81.66	9.26
11	Eucalyptus	15.00	75.93	30.00	66.66
12	Besrum	46.66	25.14	90.00	0.00
13	Neem	51.66	17.11	90.00	0.00
14	Karanj	46.66	25.14	90.00	0.00
15	Datura	36.66	41.18	64.16	28.71
16	Control	62.33		90.00	
	S Em±	3.14		1.23	
	CD (5%)	9.1		3.6	

* Means of three replications

** Days after inoculation

Table 2. Evaluation of medicinal oils against *Rhizoctonia solani in-vitro* condition

Medicinal oils	1 DAI**		2 DAI**		3 DAI**	
	Mycelial growth (mm)*	% inhibition	Mycelial growth (mm)*	% inhibition	Mycelial growth (mm)*	% inhibition
Alsi	22.00	45.45	50.00	44.44	78.66	12.60
Til	24.33	39.67	51.66	42.60	82.66	8.15
Neem	5.33	86.78	25.33	71.85	45.66	49.26
Eucalyptus	0.00	100.00	0.00	100.00	0.00	100.00
Arandi	25.00	38.01	53.00	41.11	90.00	0.00

Mahua	7.00	82.64	31.33	65.18	53.33	40.74
Karanj	19.00	52.88	40.33	55.18	63.66	29.26
Mustatd	24.00	40.49	56.33	37.41	76.66	14.82
Control	40.33		90.00		90.00	
S Em±	1.47		0.62		1.74	
CD (5%)	4.4		1.9		5.2	

* Means of three replications
** Days after inoculation

Table 3. Effect of *Trichoderma spp* on mycelial growth of *Rhizoctonia solani*

Trichoderma species	**Dual culture (mycelial growth mm)***		**% Inhibition**
	*Trichoderma**	*Rhizoctonia **	
Trichoderma viride	60.66	29.34	67.40
Trichoderma harzianum	67.33	22.67	74.81
Trichoderma spp (Mushroom isolates)	52.00	38.00	57.77
Control	90.00	90.00	
CD (5%)	2.3	2.3	
S Em±	0.70	0.70	

*Mean of three replication

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RESEARCH ARTICLE

ACID-RAINS ITS CAUSES AND IMPACTS ON CROPS IN NCR REGION

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Abstract: Most recently (2014-2015) the author has gone through vigorous survey of the literature and observed a very challanging natural climatic havoc, hazards to disturb the invironment which may cause discomfort to whole of the humanity through out the world. The investigater who recently studied the composition of rainwater from 5 selected locations, the result is worried that it is pouring 'acid rain', caused by vehicular, industrial pollution and frentic urbanisation. ''In Pune and Nagpur, the amount of acid in rainwater has gone up five times since 1995,'' said V.K. Soni[1], a senior meteorologist with the Indian Meterological Department (IMD) who conducted the study with colleague Jayant Sarkar, Former director of the IMD's air pollution unit. The researchers also collaborated with the World Meteorological Organisation.

Keywords: Acid rains, Crops, NCR region

INTRODUCTION

Samples of rainwater from Pune, Nagpur, Vishakhapatnam, Srinagar, Allahabad, Jodhpur, Kodaikanal, Minicoy (Lakshadweep) Mohanbari and Port Blair were tested at the IMD, Pune. The results indicated high amounts of sulphur dioxide and nitrogen oxide-major contents of acid rain-that are emitted into the atmosphere from vehicles, coal-fired power plants and industries.

The US Environmental Protection Agency (EPA) website says polutatnts in acid rain interact in the atmosphere to form fine sulphate and nitrate particles that can be ''transported long distances by winds and inhaled deep into people's lungs. Some particles can also penetrate indoors.' Some medical practicioners say acid rain is linked to the neurodegenerative Alzheimer's Disease.

''Drinking acidic rainwater directly or consuming vegetables grown on water containing acidic or metallic element can cause Alzheimer's disease,'' maintained Nagpur-based neurologist Rajeev Deshpande.

''When there is a decline of 1 pH, it means acidity has gone up by 10 times,'' said Soni. ''In Nagpur, acidic elements in rainwater were 4.7 pH during 2005-06 as against 5.6 in 1995.''

More recently the scientists of Indian Institute of Tropical Metrology (IIMT) Puna have carried out the research on the acid rains. During the years 2003-2005, the eight places of National Capital Region (NCR). Bulandsahr, Garh Mukteshawar, Murad nagar, Saradhana, Panipat Charkhi-Dadri, Hodal and Behrod were selected and 355 Samples of rainy water at the height of 10-15 meters from earth surface were collected with the help of the instruments borrowed from Stockhom University Sweedan during monsoon season. Scientists, **Suresh Tiwari *et al.*[2]** Observed that out of the eight places, only in three places Panipat 31, Sardhana 29 and Murad Nagar 12 Percent the rainy-water contaminated with acid. The presence of acid in rainy-water is due to the high quantity of sulphate and nitrate in the air. Surprisingly, the NCR rainy-water also contains sea salt inspite of the fact that the distance between NCR and the sea-shore is 1100 meter.

Causes of acid-rain

The rainy-clouds are present at a height of about 5.0 Kilometers from the surface of the earth, whilist the layers of particles suspended in the atmosphere are about 2-3 kilometers. In the rain, these suspended particles assimilate in the rainy-drops and the Sulphate particles form sulphuric acid and nitrate particles form nitric acid in the atmosphere in more and then gradually decreases. The rate of rise of the sulphate particles in the air is due to the smoke of combustion of coal coming out from the chimnneys of the factorises and brick-kilns and that of nitrate particles is caused by the vehicular smoke, besides these sources, the author is of the view that in the N.C.R. region, the thermal powerplants, small scale industries, brick kilns are also responsible for the production of these gases.

RESULT AND DISCUSSION

The author selected 5 locations, Faridpur, Dankaur, kasana, Dadri and Arthala spread over N.C.R. region. Samples of Soil and Water from traditional wells and ponds were collected employing the suitable methods as described in the literature before first monsoon showers during the period May 2016 to June 2016 and after heavy rains during the period July to September 2016. The investigater observed that before first monsoon shower the pH of the Soil found in the range of 9-10.0 and that of water from traditional wells and ponds in the range of 7.2 to 6.8. Contrary to these observations the values of pH during the rainy Season August to September 2016 change abruptly indicating an increase in hydrogen

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ion concentration in the soil and water. The results are tabulated in Table -1.

Table 1. pH values of Soil and Water in N.C.R. Region.

Locations	Pre Monsoon May- June 2016			Post Mansoon July-September 2016		
	Soil	Traditional Wells	Ponds	Soil	Traditional Wells	Ponds
1. Faridpur	9-10	7.2	7.5	6.8	4.7	4.5
2. Dankaur	8.5-9	6.8	7.8	6.2	5.2	5.8
3. Kasama	8-9.2	7.6	7.6	5.95	4.2	4.6
4. Dadri	8.5-9.2	7.9	7.8	5.5	5.5	4.8
5. Arthala	9.2-10	7.5	9.0	6.3	4.6	4.2

The results show that pre-monsoon season the nature of soils is basic and that of traditional wells and ponds are some what neutral where as post mansoon season, soils appear to be acidic and that of traditional wells and ponds are more acidic in nature. The plausible explanation may be put forworth as during monsoon, the acids are formed in the atmosphere which penetrate in the earth crest and the soil becomes acidic and that the composition of water of traditional wells and ponds changes and becomes acidic A schemetic diagram of acid rain, the formation of H_2SO_4, HNO_3 and Sulphate particles in the atmosphere is given in Fig. 1.

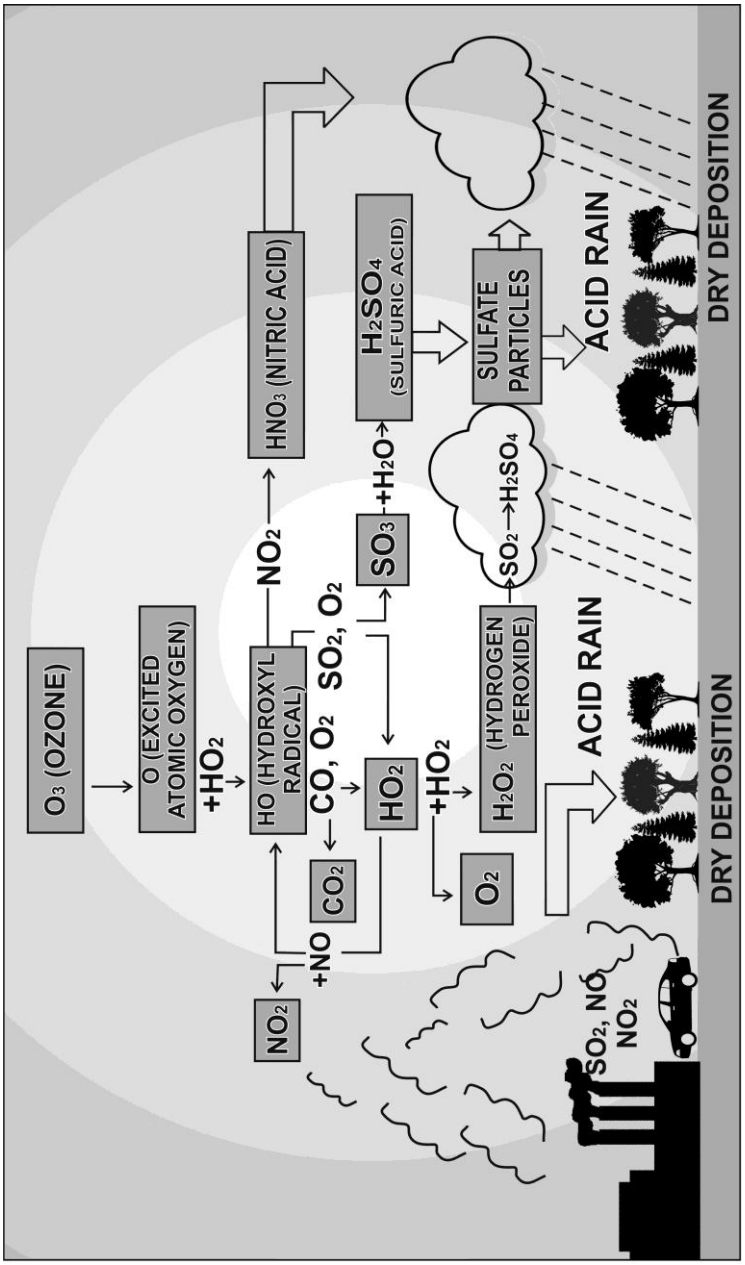

Fig. 1

Impact of acid-rain on crops and health

The acid rains may cause the following painful results.

1. When the percentage of acid in rainy-water is more than 2-3, then the grains of the standing crops in the fields may damage, as they become black-spotted and delayed.
2. If it is raining for a long period, the soil becomes more acidic causing there by a decrease in the soil fertility, which may affect the germination of the seeds and crops-yields to a great extent and thus the soil will tend to become gradually, acidic affecting fertility of the soil.
3. The author has collected a report from the farmers of the said region regarding the impacts of acid rains on kharif crops. The mayjority views are the germination of seeds is not upto the mark, the leaves of the plants are found to be black spotted and weak. Because of these impacts, the yields are poor and hence adversely may affect the economic conditions of the farmers.
4. There is a possibility of some skin diseases particularly in taking bath and wet with acid-rains.
5. Acid-rains pollute the water of traditional wells, ponds and canals which may be harmful for drinking and may cause the infections in the lungs and intestine.
6. The regions which are affected with the acid-rains, the climate[4] may be rich in sulphate and nitrate elements, the inhabitants may suffer many diseases like Asthama, Cancer and other related to breathing. Also, If the soil contaminated with acid-rains is used for the manufecture of bricks, then the structures built with these bricks will be weak.

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