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Role of VAM in alleviating allelopathic stress of *Parthenium hysterophorus* on maize (*Zea mays* L.)

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Abstract

Experiments were conducted to evaluate the impact of allelopathic potential of *Parthenium hysterophorus* on germination, growth and yield of maize (*Zea mays* L.) as well as the role of Vesicular Arbuscular Mycorrhizae (VAM) in alleviating allelopathic stress. In the first experiment germination and seedling growth response of maize to 5, 10, 15, 25 and 50 % w/v aqueous extract of *P. hysterophorus* was studied in petri dishes. Higher concentrations of 25 and 50% aqueous extract of Parthenium significantly reduced the germination of maize grains. Root and shoot growths of seedlings were also similarly affected. In the other experiment, shoot material of *P. hysterophorus* was cut into very small pieces and mixed in the heat sterilized pot soil @ 0, 5 and 10 % w/w, half the pots were inoculated with VAM. The results regarding the various vegetative and reproductive growth parameters revealed that the maize crop was not susceptible to applied rates of *P. hysterophorus* Mulch, the maize growth was considerably enhanced in 5 % treatment while in 10 % mulch treatment crop growth was as good as in control while plants inoculated with VAM showed markedly enhanced the crop growth both in control as well as *Parthenium* mixed treatments. Mycorrhizal colonization was markedly suppressed by mixing shoot material of *P. hysterophorus* at vegetative growth stage especially in 10 % treatment.

Key words: *Parthenium hysterophorus*, maize, vesicular arbuscular mycorrhizae, allelopathy.

Introduction

Parthenium hysterophorus Linn. is a member of the Compositae (Asteraceae) family. It is an annual wasteland weed growing naturally along roadsides, wastelands and also in crop fields in East and South Africa, India and Australia, South America, West Indies, Mexico, Southern China, Pacific Islands, Vietnam, Cuba, and Canada. (Towers *et al*., 1977). The weed did not find any place in the list of world's worst weeds till 1977 (Holm *et al*., 1977) but within the last decade it has become one of the seven most dreaded weeds of the world (Singla, 1992). It entered Pakistan about a decade ago and spread and invaded rapidly the wastelands, roadsides and watercourses in the last 2-3 years (personal observations). In a very short period of time, it advanced from isolated outbreaks to establish core infestation in the Punjab province. Further, factors such as (i) the absence of natural agents that restrict the spread of this plant as in its original home, (ii) high fecundity, (iii) efficient seed dispersal mechanisms, (iv) monopolistic allelopathic impact on most other plant species, (v) unsuitability for grazing because of the presence of anti-feedants in the plant system and (vi) wide adaptability to varying soil and agroclimatic conditions have enabled this plant to invade a variety of growing environments particularly in situations associated with human activities. The weed can germinate, flower and set seeds within four weeks.

The chemical analysis has indicated that all the plants parts of *Parthenium* including trichomes and pollens contain toxins called sesquiterpene lactones parthenin that is a natural constituent of *Parthenium*. The growth regulating activity was studied in terms of morphogenetic response of hypocotyl cuttings of Mungbean, lentil and pigeon pea. At low concentration (up to 10 ppm.), a significant enhancement in rooting of hypocotyl cuttings was observed (Batish *et al*., 1999). Rajan (1973) and Kanchan and Jayachandra (1975) were the first to report the presence of plant growth inhibitors in *Parthenium* weed and latter author identified parthenin, caffeic acid, and p-coumaric acid as the primary inhibitors in stem tissues. Later, Kanchan and Jayachandra (1979) found that these inhibitors were also present in root exudates (Kanchan and Jayachandra; 1980). In addition a range of phenolics including caffeic acid, ferulic acid, vanillic acid, anisic acid, p- anisic acid, chlorogenic acid, coronopillin, coumaric acid, fumaric acid and parahydroxy benzoic acid are lethal to human beings and animals (Mahadevappa, 1997; Oudhia and Tripathi, 1998). The phenolics found in *Parthenium* also inhibit the

germination and growth of several crop plants and multipurpose trees (Dharmaraj and Ali, 1985; Shrivastava *et al*., 1985; Dayama, 1986). Yield decline by 40% per cent in agricultural crops (Khosala & Sobti, 1981) and 90 per cent reduction in forage production has been reported (Adkins *et al*., 1997).

Vesicular arbuscular mycorrhizae (VAM) are the most common underground symbiosis between plant roots and fungi. These fungi are known to enhance the crop growth and yield, uptake of water and essential mineral elements, photosynthesis, symbiotic nitrogen fixation and crop defense mechanism against a variety of stresses in terrestrial plants (Jefferies, 1987). Recently some workers have reported that inoculation of VAM increases the crop tolerance against allelopathy and enhance crop growth under this stress (Bajwa *et al.,* 1999; Javaid and Bajwa, 1999).

The present study was undertaken to investigate the effect of aqueous extracts and mixing of *P. hysterophorus* on germination and growth of maize and role of VAM inoculation on crop growth and yield in maize under an allelopathic stress.

Materials and Methods

Experiment 1:

Freshly taken aerial portions of *P. hysterophorus* were crushed with the help of pestle and mortar and were soaked for 48 hours in sterilized water at the rate of 50g of *Parthenium* per 100ml of sterilized water. This 50% w/v aqueous extract of *Parthenium* was used as stock solution to prepare various test concentrations viz: 5, 10, 15, 25, and 50 % by adding sterilized water.

Healthy seeds of maize were surface sterilized and placed in sterilized petri dishes having double layer of filter papers, moistened with different aqueous extracts. Control received sterilized water only. Three replicates with 10 seeds each were used for each treatment. Emergence of radical was taken, as criterion for germination and percentage germination was recorded upto 8 days .The effect of allelopathic extract on early growth of maize was evaluated in terms of root/shoot fresh and dry weights and lengths.

Experiment 2:

Clay pots of 30 cm diameter were filled with sieved and heat sterilized field soil. Each pot contained 10 kg of soil. The shoots of *P. hysterophorus* were cut into very small fragments and were mixed in pot soil @ 5 and 10 % w/w. The pots were kept in wire netting house under natural conditions and irrigated with tap water. The pots were left for 15 days for decomposition of materials.

For VAM spore inoculum, endogonaceous spore extraction was carried out following wet sieving and decanting technique of Gerdemann and Nicolson (1963). Mycorrhizal spore inoculum in pots was employed 15 days after mulch treatment, following funnel technique of Menge and Timer (1982). For this purpose paper funnels of about 90 mm diameter were prepared and buried in the center of the experimental pots (mycorrhizal sets) and filled with sterilized soil. While filling was being carried out, spore inoculum at the rate of 250-300 spores, was introduced in 2 layers. Thus the inoculum placement consequently was kept at 3cm and 5cm depth from the top downwards. After mycorrhizal application, 6 surface sterilized and pre-soaked maize seeds (for rapid germination) were sown in each pot, in funnel at the depth of 1cm. After one week of germination, the number of seedlings per pot was reduced by careful manual thinning and only 3 uniform and healthy seedlings were left in each pot. Each treatment was replicated thrice. Plants were harvested 30, 90 and 120 days after sowing. At the time of each harvest, plants were uprooted carefully so that their roots were not damaged. The plants were washed under tap water to remove soil debris from roots. The shoots and roots of each plant were separated. The length (cm) and fresh weight (g) of roots and shoots of each replicate were recorded.

A sub-sample of root of each sample was preserved in FAA (Formalin, glacial acetic acid, alcohol in the ratio of 5:5:90 by volume) for mycorrhizal study. For dry weight determinations, shoots and remaining roots were oven-dried at 70˚C for 48 hours and re-weighed. Cobs from each replicate plant of various treatments at the final harvest were separated and their number, fresh and dry weights were determined.

Fixed root samples for all treatments were cleared and stained for study of VAM colonization, following modified technique of Phillips and Hayman (1970).

Data regarding the various plant growth and VAM parameters were analyzed statistically by applying Duncan's Multiple Range Test (Steel and Torrie, 1980) and t-test.

Results

Experiment 1:

Maximum germination of 90% was achieved in control after 2 days of incubation. No further enhancement in germination was recorded thereafter (Fig. 1). The various aqueous extracts of

Parthenium affected the germination of maize differently. In 5% extract, the germination percentage of maize after an initial decline enhanced continuously and achieved 6% greater germination. However, the difference was insignificant as compared to control. The presence of 10% extract caused a small insignificant decline in germination. The maximum inhibition was recorded in 15% extract (Fig. 1) in which a loss of 30% in germination was consistent. The adverse effect of 25 and 50% extract was also significant (P = 0.05) as compared to control and caused 10-15% inhibition in germination (Fig. 1).

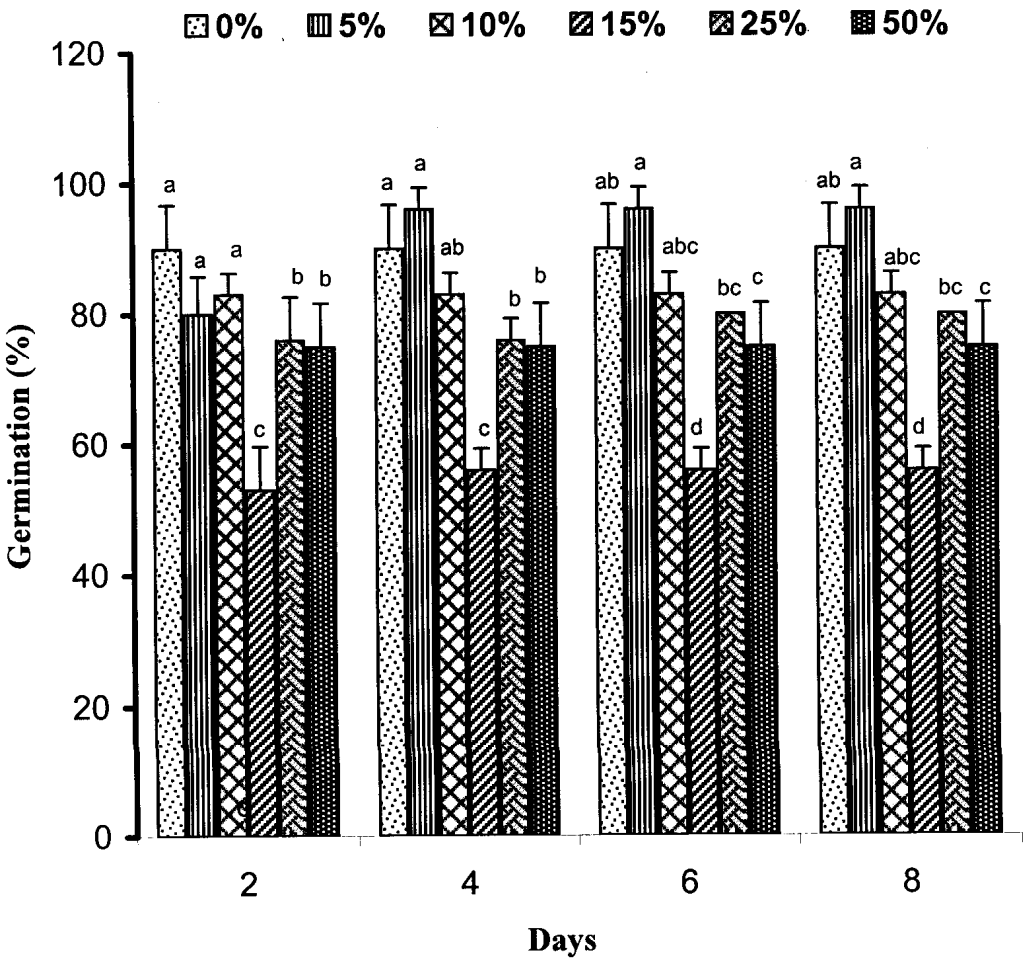

Fig.1: Effect of aqueous extracts of *Parthenium hysterophorus* on germination of maize. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference (P = 0.05) as determined by DMR Test.

In all corresponding extract concentrations, a depression parallel to shoot length was recorded in shoot fresh biomass production. The negative impact of 25 and 50% extract was more pronounced although insignificant as compared to control (Fig 2-B). The dry biomass production response of maize seedling to various aqueous extracts of *Parthenium* was similar to that of fresh weight (Fig 2-C).

Root length of maize, in general, was adversely affected by aqueous extracts of *Parthenium*. The effect was found to be variable with respect to different concentrations of aqueous extracts (Fig 3-A). Lower concentrations of extracts *viz* 5-15% were less inhibitory as compared to higher concentrations of 25 and 50%. Effect was significant (P=0.05) in 5, 25 and 50% extracts. Maximum inhibition of 88% in root length was recorded in 50% followed by 25% extract of *Parthenium* (Fig 3-A). The effect of different concentrations of aqueous extract of *Parthenium* on root fresh and dry biomass was

different to that of root length (Fig 3) i.e. suppression was observed on lower concentration of 5% and higher concentrations of 25% and 50%. However an enhancement in biomass production was recorded in 10% after 90 days of sowing i.e., at flowering stage (Fig 3C).

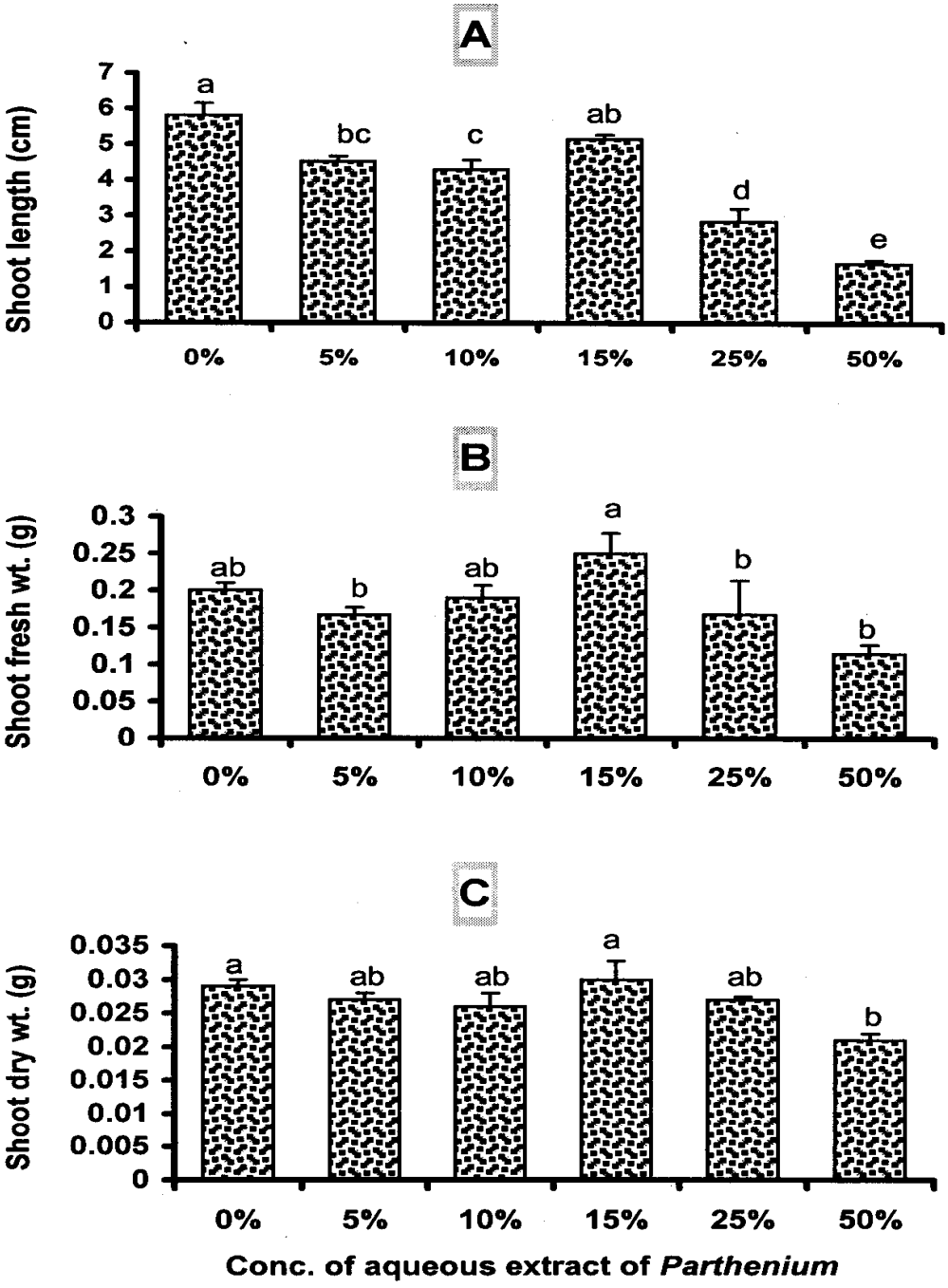

Fig.2 (A - C): Effect of aqueous extracts of *Parthenium hysterophorus* on shoot growth of maize in aqueous extract bioassay. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference (P = 0.05) as determined by DMR Test.

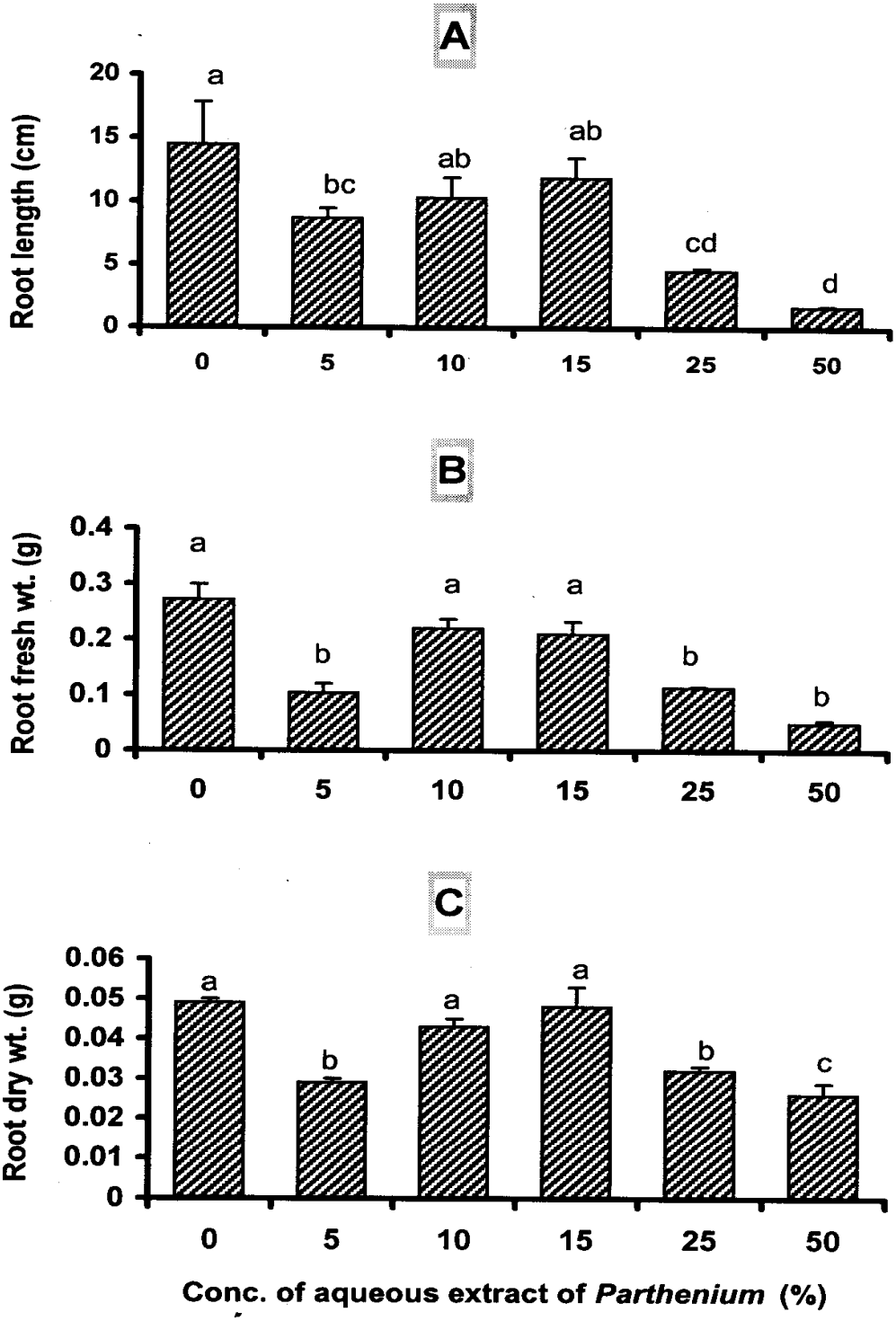

Fig. 3 (A - C): Effect of aqueous extracts of *Parthenium hysterophorus* on root growth of maize in aqueous extract bioassay. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference (P = 0.05) as determined by DMR Test.

Experiment 2:

Effect of *Parthenium* residue mixing and mycorrhizal inoculation on shoot growth:

In non- mycorrhizal treatments, *Parthenium* residue mixing of 5 and 10% w/w enhanced shoot length in maize at vegetative stage i.e. 30 days after sowing. Effect was significant (P = 0.05) in 5% treatment as compared to control (Fig 4A). At later growth stages of flowering and maturity i.e. after 90 and 120 days of sowing, shoot length in 5% treatment was also significantly greater as compared to control (Fig. 4 A&B). The mycorrhizal inoculation failed to induce any noticeable effect, in terms of shoot length, at vegetative stage. However, an increase in length was recorded at later two growth stages. The mycorrhizal association provided more pronounced effect in 5% as compared to 10% treatment and was significant (P = 0.05) at maturity as compared to control and 10% treatments (Fig 4 A-C).

Shoot biomass production in non-mycorrhizal treatments was not affected considerably by *Parthenium* mixing at the vegetative stage. However in 5% treatment, an insignificant enhancement was evident (Fig 5 A). From vegetative to flowering stage, the comparative rate of dry biomass increments improved in control and 10% treatments. The response of *Parthenium* mulch was, therefore, not evident. However, after 90 days, the results showed the same trend as after 30 days (Fig 5 A-C). Comparative analysis among non-mycorrhizal and mycorrhizal treatments revealed a significant difference (P = 0.01) in control due to mycorrhizal inoculation only at vegetative stage. In *Parthenium* mixed treatments effect of mycorrhizal inoculation was insignificant (Fig. 5A-C).

Effect of *Parthenium* residue mixing and mycorrhizal inoculation on root growth:

Root length in *Parthenium* mixed treatments was insignificantly different from control at all the three growth stages. Root length did not show any pronounced response to mycorrhizal inoculation at vegetative and flowering stage. However, at maturity a significant increase was recorded in control (P = 0.05) and 5 % *Parthenium* mixed (P = 0.01) treatment (Fig. 6 A - C).

The data obtained on average dry weight measurements (Fig 7A-C) displayed that the dry biomass production in non-mycorrhizal plants was stimulated in 5% treatment as compared to control. Effect was significant (P = 0.05) at maturity (Fig. 7 A-C). Mycorrhizal inoculation enhanced root biomass production both in control and *Parthenium* mixed treatments at all the three growth stages. Effect was significant in 10 % treatment at flowering stage and in 5 % treatment at maturity (Fig. 7 A- C).

Effect of *Parthenium* residue mixing and mycorrhizal inoculation on yield:

In non-mycorrhizal *Parthenium* mixed plant, an insignificant (P = 0.05) difference was observed in all three treatments at three growth stages. A pronounced enhancement in dry biomass of cob was observed in 5% treatment (Fig. 8). This pattern in mycorrhizal condition was exactly same as in non-mycorrhizal treatments with respect to dry weight gain of cobs. A marked enhancement in 5% and slight depression in 10% was observed in contrast to control (Fig.8). As far as non-mycorrhizal and mycorrhizal treatments are concerned, more pronounced advantage, in terms of weight gain of cobs, due to inoculation was achieved in control and lowest in 10% *Parthenium* mixed treatment. However, the difference among mycorrhizal and non-mycorrhizal treatments, in general, was insignificant statistically (Fig. 8).

Effect of *P. hysterophorus* mixing on VAM infection in maize:

In general the mulch treatment induced insignificant effects on VAM infection. However some enhancement had been observed in arbuscules and vesicles, in 5% mulch plants. The relevant observations are as follows:

A marked enhancement in number of arbuscules was observed at vegetative stage. i.e., 30 days after sowing. The number gradually decreased, as concentration of mulch increased from 5 to 10% (Fig. 9-A). Ten percent *P. hysterophorus* residue mixing caused significant (P = 0.05) depression in arbuscular colonization as compared to control. In the following stage i.e. flowering stage, a significant (P = 0.05) enhancement was recorded in 5% treatment with respect to control. In 10% mulch treatment, the rate of arbuscular formation was not improved very markedly (Fig.9 B). At the final growth stage, the effect of *P. hysterophorus* residue mixing was more pronounced and arbuscular formation was declined sharply in both 5 and 10% mixing. However, the decline was statistically significant (P = 0.05) in 10% treatment in comparison with control (Fig. 9A-C).

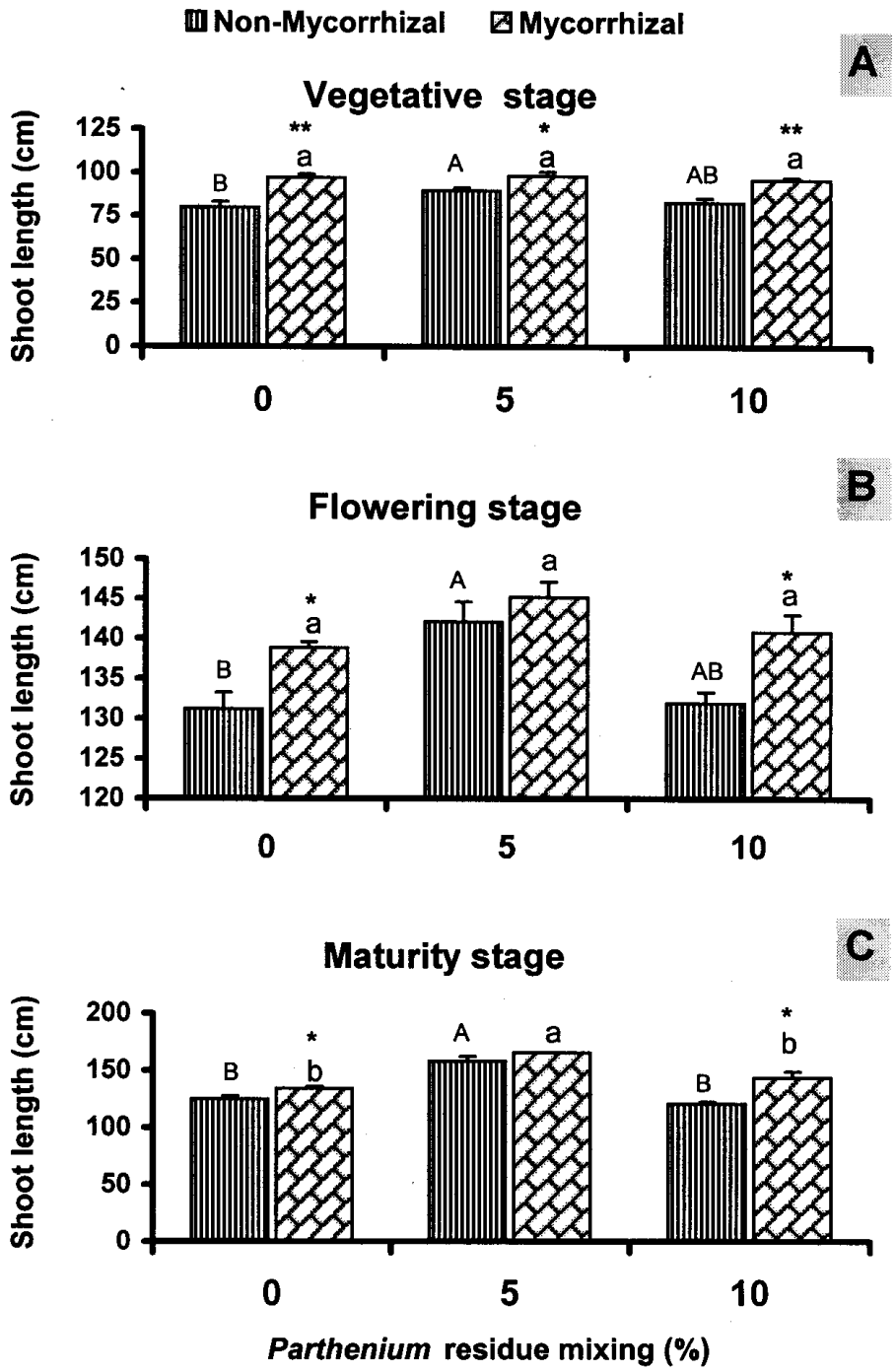

Fig. 4 (A - C): Effect of *Parthenium* residue mixing and mycorrhizal inoculation on shoot length of maize. Vertical bars show standard errors of means of three replicates. Values with different capital and small letters show significant difference (P = 0.05) among non-mycorrhizal and mycorrhizal treatments, respectively as determined by DMR Test. *,**,Show significant difference between two corresponding mycorrhizal and non-mycorrhizal treatments at 5, and 1% level of significance, respectively,as determined by t-test.

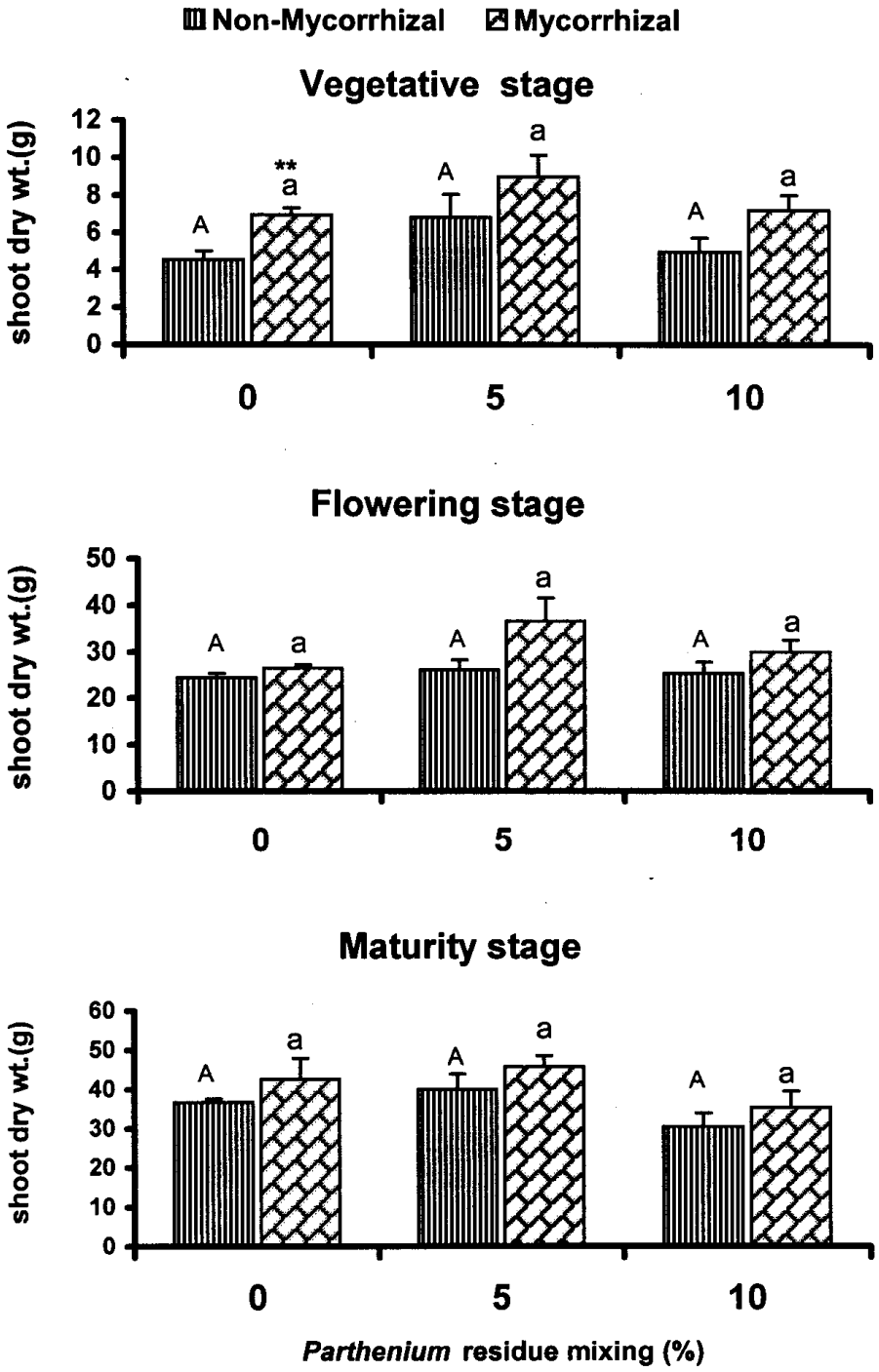

Fig. 5 (A-C): Effect of *Parthenium* residue mixing and mycorrhizal inoculation on shoot dry wt. of maize. Vertical bars show standard errors of means of three replicates. Values with different capital and small letters show significant difference (P = 0.05) among non-mycorrhizal and mycorrhizal treatments, respectively as determined by DMR Test.
**, shows significant difference between two corresponding mycorrhizal and non-mycorrhizal treatments at 1% level of significance, respectively, as determined by t-test.

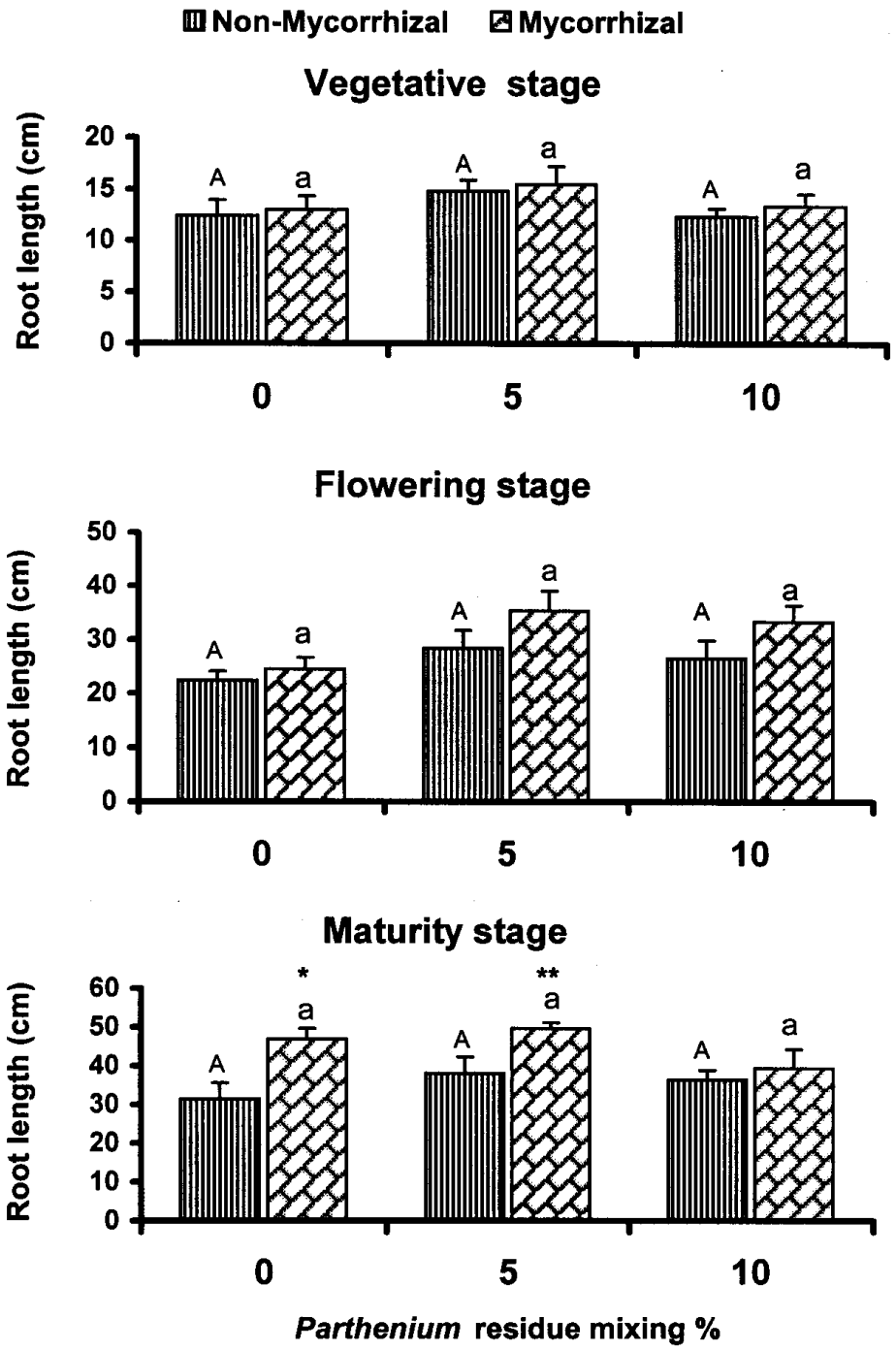

Fig. 6 (A-C): Effect of *Parthenium* residue mixing and mycorrhizal inoculation on root length of maize. Vertical bars show standard errors of means of three replicates. Values with different capital and small letters show significant difference (P = 0.05) among non-mycorrhizal and mycorrhizal treatments, respectively as determined by DMR Test. *,**, show significant difference between two corresponding mycorrhizal and non-mycorrhizal treatments at 5 and 1% level of significance, respectively, as determined by t-test.

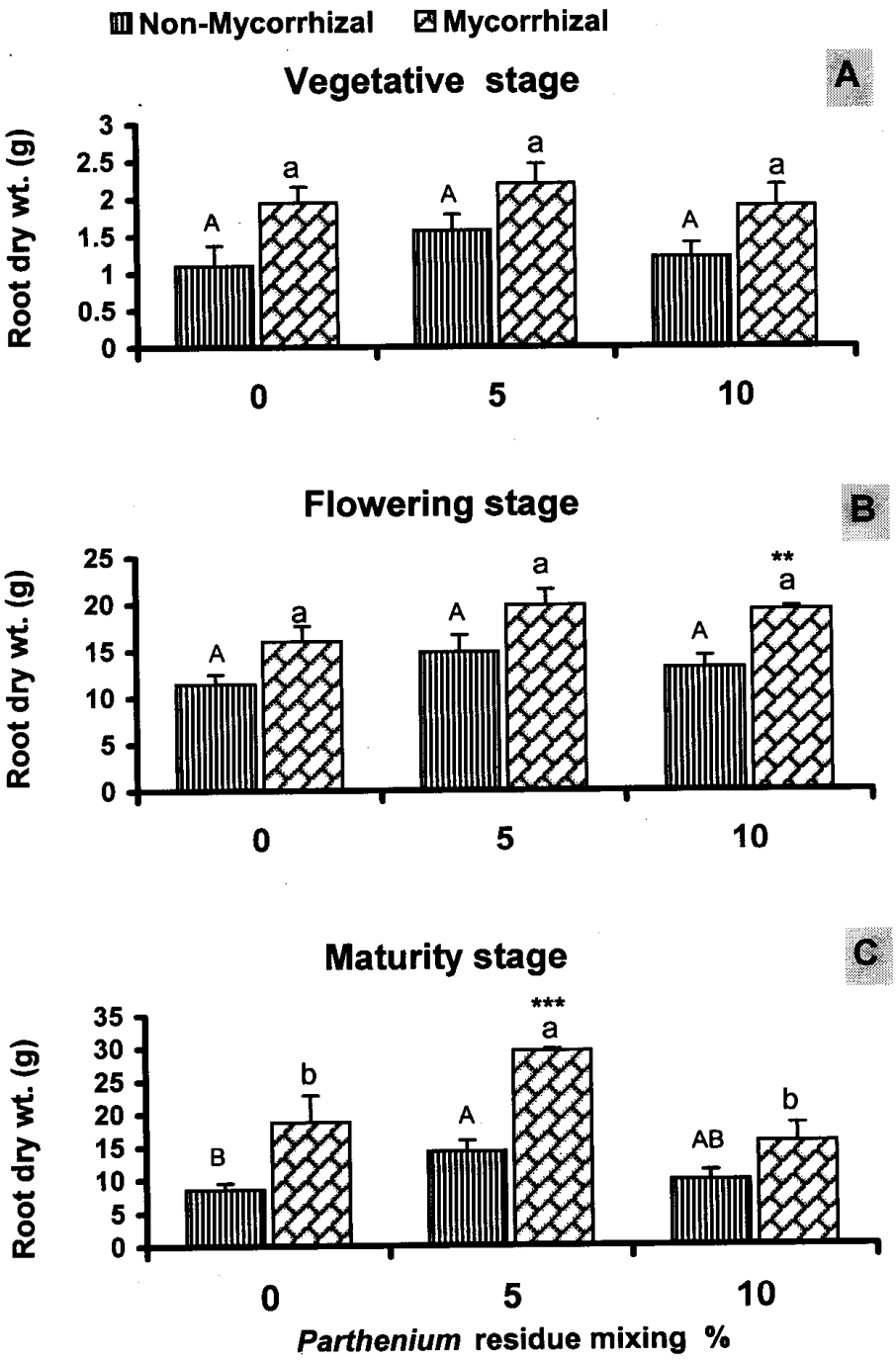

Fig. 7 (A-C): Effect of *Parthenium* residue mixing and mycorrhizal inoculation on root dry wt. of maize. Vertical bars show standard errors of means of three replicates. Values with different capital and small letters show significant difference (P = 0.05) among non-mycorrhizal and mycorrhizal treatments, respectively as determined by DMR Test. **,***,show significant difference between two corresponding mycorrhizal and non-mycorrhizal treatments at 1and 0.1% level of significance, respectively,as determined by t-test.

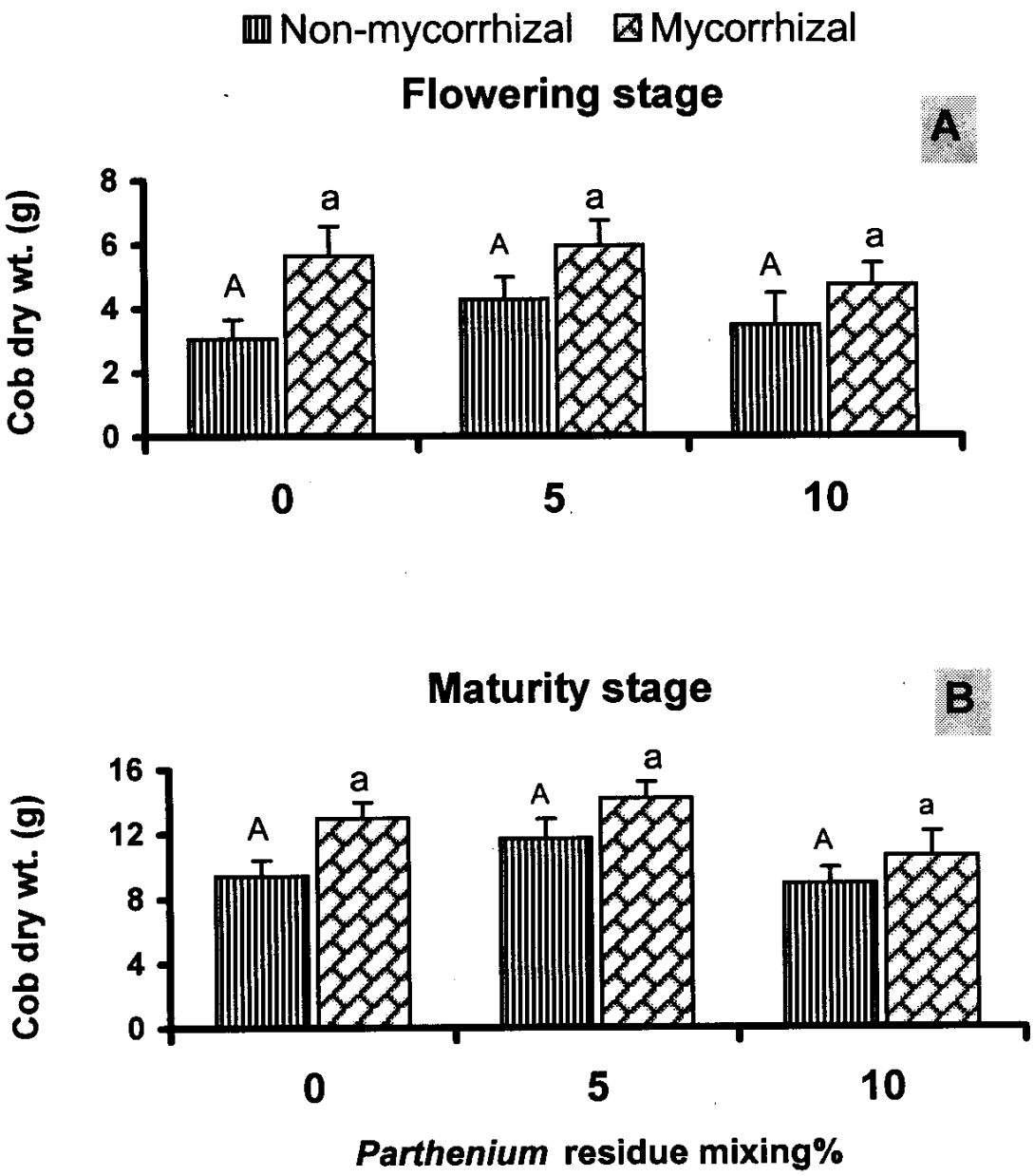

Fig.8 (A & B): Effect of *Parthenium* residue mixing and mycorrhizal inoculation on cob dry wt. of maize. Vertical bars show standard errors of means of three replicates. Values with different capital and small letters show significant difference (P = 0.05) among non-mycorrhizal and mycorrhizal treatments, respectively as determined by DMR Test. There is an insignificant difference between two corresponding non-mycorrhizal and mycorrhizal treatments as determined by t-test.

As far as vesicle formation is concerned, the control treatment did not seem to follow typical pattern of mycorrhizal colonization, whereby vesicle formation in general increased towards host maturity. The usual pattern was rather followed in residue mixed treatments (Fig. 9A-C). Consequently, the vesicle formation gradually increased from vegetative to maturity stage both in 5% and 10% treatments and it was significantly (P = 0.05) high in 5% than their counter parts in control as well as 10% treatments (Fig. 9A-C).

The assessment of VAM infection in sterilized soil with and without mulch revealed that mulch greatly suppressed VAM colonization in initial stage in 10% treatment. While the effect was less pronounced and insignificant in 5% mulch treatment as compared to control (Fig.10 A). The extent of VAM gradually increased in later 2 stages following the same trend of growth as in vegetative stage. However, at final stage i.e., after120 days a marked increase in mycelial colonization was evidenced in 10% mulch treatment resulting in values parallel to control (Fig. 10 C). The extent of VAM colonization was found to be slightly depressed in 5%, the difference being statistically insignificant.

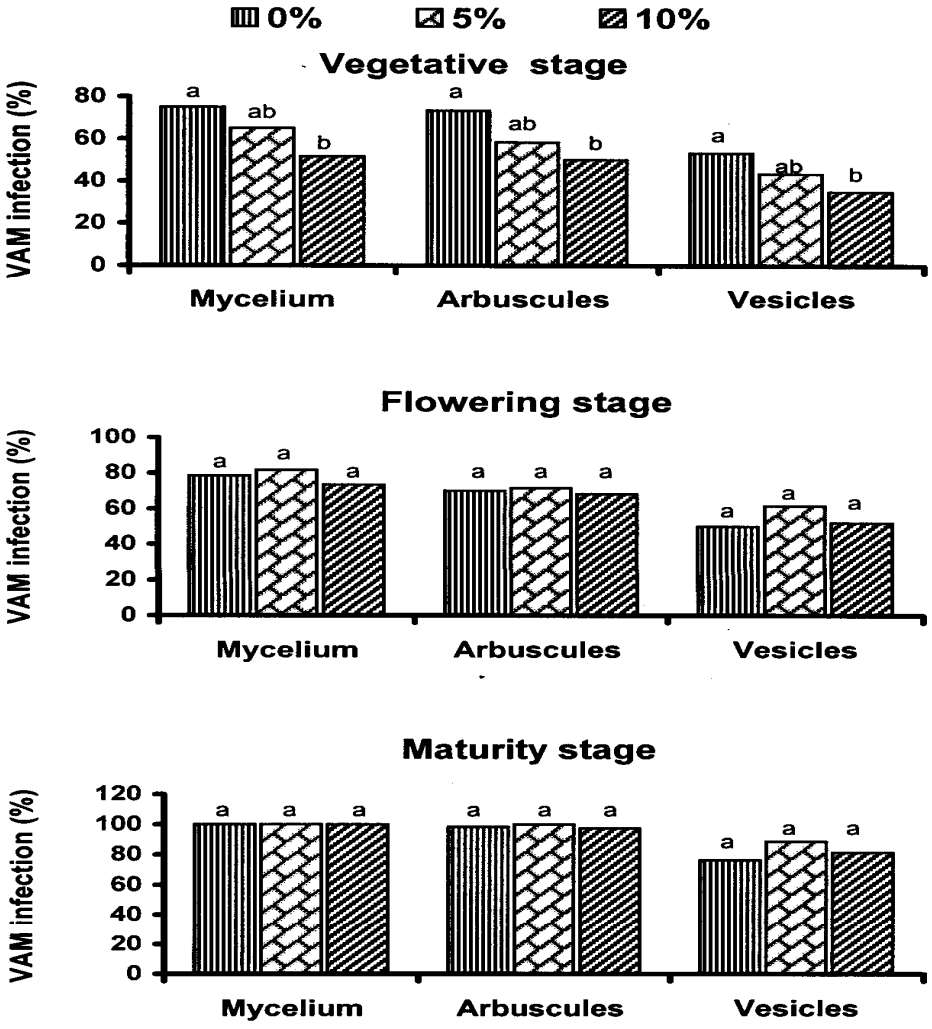

Fig. 9 (A-C): Effect of different concentration of *Parthenium* residue mixing on percentage mycelial, arbuscular and vesicular infection in maize. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference (P = 0.05) as determined by DMR Test.

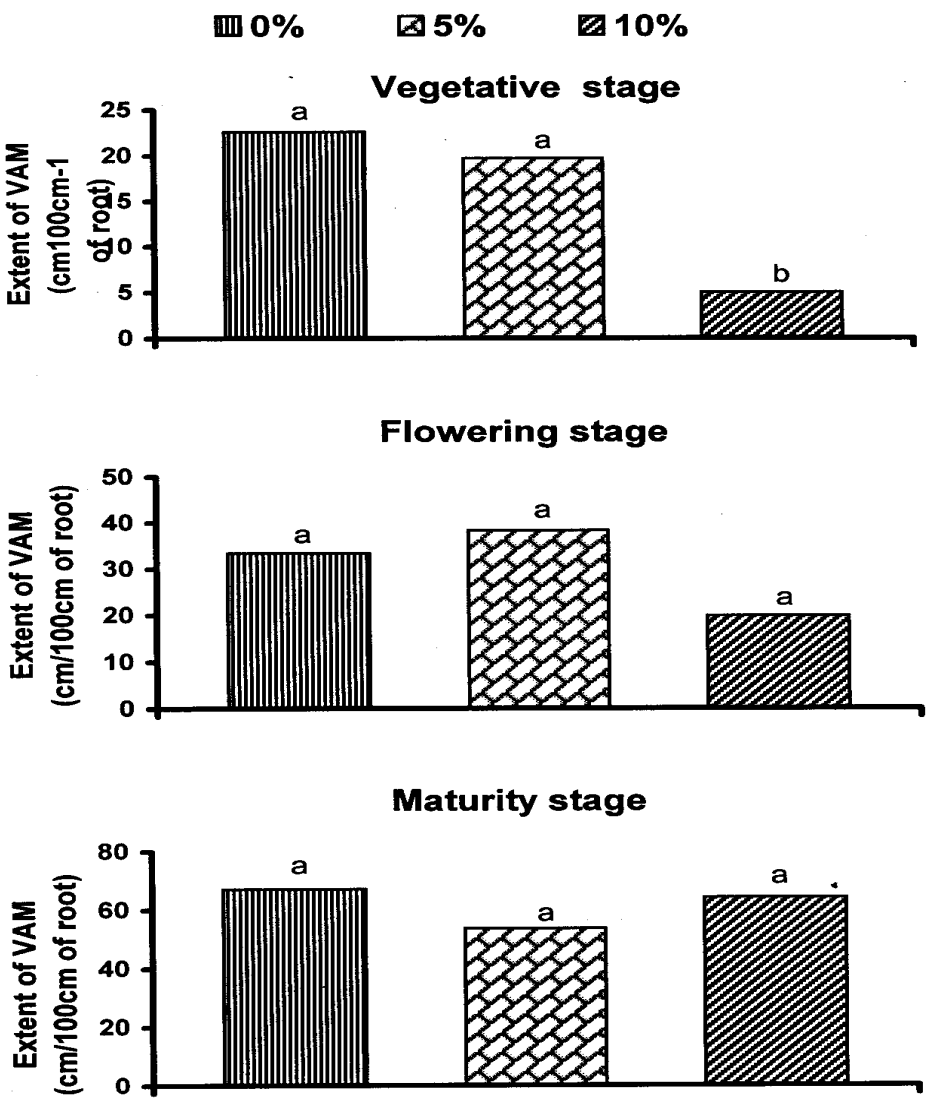

Fig.10 (A-C): Effect of different concentration of *Parthenium* residue mixing on extent of VAM in maize. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference (P = 0.05) as determined by DMR Test.

Discussion

The investigations carried out presently to discern allelopathic potential of *P. hysterophorus* revealed rather contrasting results. In the case of aqueous extract bioassays, both germination and early growth of maize was greatly depressed particularly in response to higher concentrations. No growth inhibition was evidenced when *P. hysterophorus* was employed in mulch treatments. 5% concentration either used as aqueous extract or in mulch was found to induce growth stimulation. The mycorrhizal plants, in general, attained better growth than non-mycorrhizal plants.

In aqueous extract bioassay, the germination of maize was reduced by 20-50% due to aqueous extracts of *P. hysterophorus*. A slight enhancement has been shown in 5% concentration but about 50% reduction was observed in 50% aqueous extract concentration. Similar reduction in germination has also been observed in other crops due to allelopathic plant extracts (Hussain and

Abidi, 1991; Noor and Khan, 1994). However, Narwal (1994) has reported a stimulatory effect of lowest concentration (5%) of allelopathic extracts on germination.

The seedling growth was also reduced by aqueous extracts. Similar phytotoxic effects of plant species like *Brassica napus* (Choesin and Boerner, 1991), *Rorippa sylvestris* (Mizutani, 1989) and *Ipomea tricolor* (Anaya *et al*., 1990) are also known. The reduced seedling growth could possibly be attributed to reduce cell division due to presence of phytotoxins in the environment (Muller, 1965). The inhibitory potential of *Parthenium* extracts increased with increasing concentrations of the extract. These findings are in line with the observations of earlier investigations of Lawrence *et al* (1991) and Noor and Khan (1994). Root growth was observed to be more sensitive to aqueous extracts than shoot growth. Other workers have also reported the reduced root growth in response to allelochemicals (Noor and Khan, 1994).

The negative (stimulatory) allelopathic effects of aqueous extracts of *Parthenium* have earlier been reported on field crops by Oudhia and Tripathi, (1999). In contrast to that many allelochemicals e.g. parthenin, p-coumarioc acid, caffeic acid, coronopillin acid and sesquiterpene lactones from aqueous extract of *Parthenium* have been shown to cause positive (inhibitory) allelopathic effects (Narwal, 1994). In several studies on allelopathic weeds it is evident that germination and seedling vigour of many agricultural crops like rice, wheat, maize, groundnut etc. is not affected and these can rather be employed as green growth promoters (Oudhia and Tripathi, 1999).

In contrast to aqueous extract, no inhibition was evidenced in the growth of maize due to *Parthenium* mulch. *Parthenium* mulch treatment of 5% conversely caused a considerable enhancement. Mulching at the rate of 10% although did not induce any significant enhancement; no negative impact on growth was evidenced. These findings are supported by earlier investigations of Oudhia and Tripathi (1999) who have reported that 5% concentration of *Parthenium* leaves promoted the early germination and vigor of rice seeds. This may be attributed to the fact that varying levels of allelochemicals behave differently. The variable contents of allelochemicals of *Festuca arundinaceae* have been shown to cause variable degree of suppression in *Digitaria sanguinalis* (Peters and Zam, 1981). These results are in agreement with the previous investigations of Cronge *et al.,* (1999) who have observed in pot experiments that mulch of *Sesbania sesban* or *Morus alba* leaf material caused a significant increase in early growth and dry matter production of *Hordeum vulgare*. However the effect of higher concentrations has been shown to be inhibitory. Several other studies have reported that the increase in extract concentration produces a corresponding increase in growth inhibition (Lawrence *et al*, 1991; Noor and Khan, 1994; Ghafar *et al*., 2000) and the results have been attributed to proportional increase in phytotoxicity of the extracts. In present study however, 10% residue mixed treatment was not found to cause any inhibition in growth as compared to control. This probably indicates that the concentrations employed presently were not high enough to be effective.

Shoot growth response was comparable to that of root growth under the influence of *P. hysterophorus* mulch and a pronounced enhancement was obtained in growth. It was in line with the findings of Pervaiz (2002). A variable yield growth response of maize to VAM application was observed with respect to mulch amendments, soil sterilization and growth stages of host plant. VAM application enhanced yield growth both in treatments of 5% and 10% mulch of *P. hysterophorus* as compared to non-VAM treatments. The enhanced growth by VAM under allelopathic stress has also been reported by Bajwa *et al*., (1999).

VAM colonization was seemed to be decrease with the addition of *Parthenium* residues. This reduced VAM colonization was perhaps due to allelopathic interaction. Allelochemicals, which were leached from dead and decaying parts of *Parthenium* through irrigation, adversely affected the VAM development. Mushtaq *et al*., (1993) have reported a similar effect of *Melia azadarach* on the VAM development in maize. Similarly significant reduction in VAM development due to allelopathy of *Imperata cylindrica* has been reported by Bajwa *et al.* (1996).

The present study indicates that the aqueous extract and mixing of crop residues of *P. hysterophorus* at low concentration of 5% can probably be employed to promote early germination and growth of some crops. However this can only be recommended for crops after extensive interaction assays because allelochemicals are known to exhibit species-specific characteristics.

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