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TRIPARTITE INTERACTIONS BETWEEN ROOT LESION NEMATODE, VAM FUNGUS AND WHEAT VARIETIES

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ABSTRACT

Studies on tripartite interactions between vesicular arbuscular mycorrhiza–VAM, root lesion nematode and wheat varieties were carried out in soil pots to find out the potentiality of VAM fungus (*Glomus mosseae*) in limiting the activities of root lesion nematode (*Pratylenchus* sp.) in rhizospheres of 6 wheat varieties, while resistance / susceptibility of the wheat varieties towards the nematode / VAM fungus was also observed. On single (separate) inoculations (*G. mosseae* / *Pratylenchus* sp.) Blue silver showed positive response towards VAM fungus (85.58 ± 4.7 % infection) but least to nematode in term of diseases severity–DS (16.75 ± 6.8 %). However, the var. Mexi Pak showed susceptibility towards nematode (48.08 ± 08 % DS) and comparatively less response towards VAM fungus (55.18 ± 7.7 % infection). The combined inoculations (*G. mosseae* + *Pratylenchus* sp.) showed significant reduction ($P<0.05$) in root DS and the population of *Pratylenchus* sp. in rhizospheric regions of all wheat varieties except Mexi Pak as compared to control set of pots. Blue Silver showed maximum reduction in the population of *Pratylenchus* sp. (62.5 ± 04.71 %) while it was least in Mexi Pak (06.8 ± 05.56 %).

Key words: VAM, root lesion nematode, wheat varieties

INTRODUCTION

In Pakistan the problem of nematodes has already been recognized as one of the major threats to the agricultural yields (Maqbool, 1992). In Pakistan agriculture provides the basic means of life for more than 75 % of population where wheat (*Triticum aestivum* L.) is the staple food, cultivated in both irrigated and barani lands (Hafiz, 1986). VAM is beneficial soil fungi which universally present (Nicolson, 1967). VAM fungi improve the growth and yield of plants by increasing uptake of soil nutrients particularly phosphorus (Harley and Smith, 1983; Cooper, 1984; Hall, 1988; Wild, 1988). VAM fungi also known as the best bio-control agent against soil-borne plant pathogens including fungi, bacteria and nematodes (Stan, 1982; Jain and Hasan, 1986; Harley, 1989; Anwar and Jalaluddin, 1992; Pinochet *et al*, 1993; Sujan, 1997). The most significant aspect of VAM bio-control is that it does not entail any toxicity and disturbance of natural geo-chemicals, micro-flora and fauna of the soil. Thus it keeps soil save from chemical hazards and pollution (Cook and Baker, 1983; Lynch, 1987; Campbell, 1989).

The *Pratylenchus* species live freely in various soils as migratory endo-parasites feed on verity of cultivated and wild hosts in most parts of the world (Wiese, 1977). *G.mosseae* is commonly found in wheat and rice fields of Pakistan (Jalauddin and Anwar, 1991). This paper deals studies on tripartite interactions between VAM fungus (*G. mosseae*), root lesion nematode (*Pratylenchus* sp.) and the 6 wheat varieties (Blue Siver, Mexi Pak, Pak-70, Pavon, Sindh-83 and ZA-77) in pots soil to find out the potentiality of the VAM fungus in limiting the activities of nematode in rhizospheres of the 6 wheat varieties while the resistance / susceptibility of the 6 varieties towards the nematode and VAM fungus was also been taken into consideration. However, such tripartite interactions have not been carried out in Sindh, Pakistan. The available data of this experiment would facilitate us to be acquainted with the efficacy of VAM fungi in combating the nematodes population in soil / roots of wheat.

MATERIALS AND METHODS

Collection of soil samples: Source of primary inocula:

The primary inocula for the experiments were obtained from the soil samples collected from the agricultural field of Nawabshah district of Sindh. The collections were made by stratified random sampling plan at maturity of wheat crop from its rhizospheric regions. The soil closely adhering to root system was retained and collected in polyethylene bags of 30 cm^2 size. Each soil sample consists of three replicas each of 100 g weight collected in transparent polyethylene bags of 30 cm^2 size.

Extraction of VAM spores and nematodes from soil samples:

The VAM spores and nematodes (primary inoculua) were extracted separately from the collected soil samples by wet sieving and decanting method (Gerdemann and Nicolson, 1963) and by the method of Goodey (1957) respectively. To make sure the extracted VAM spores are viable; they were further processed by sucrose centrifugation method (Jenkins, 1964). The extracted VAM spores and nematodes were quantified on a counting dish (Southy, 1985). The extracted VAM spores and nematodes were identified morphologically by using Schenck and Perez (1990) and Willmott *et al.* (1972) respectively.

Preparation of inocula:

The primary inocula (*G. mosseae* and *Pratylenchus* sp.) extracted from the collected soil samples were multiplied and maintained separately by using the methods of Heeper (1982) and Goodey (1957) respectively for inoculation purpose.

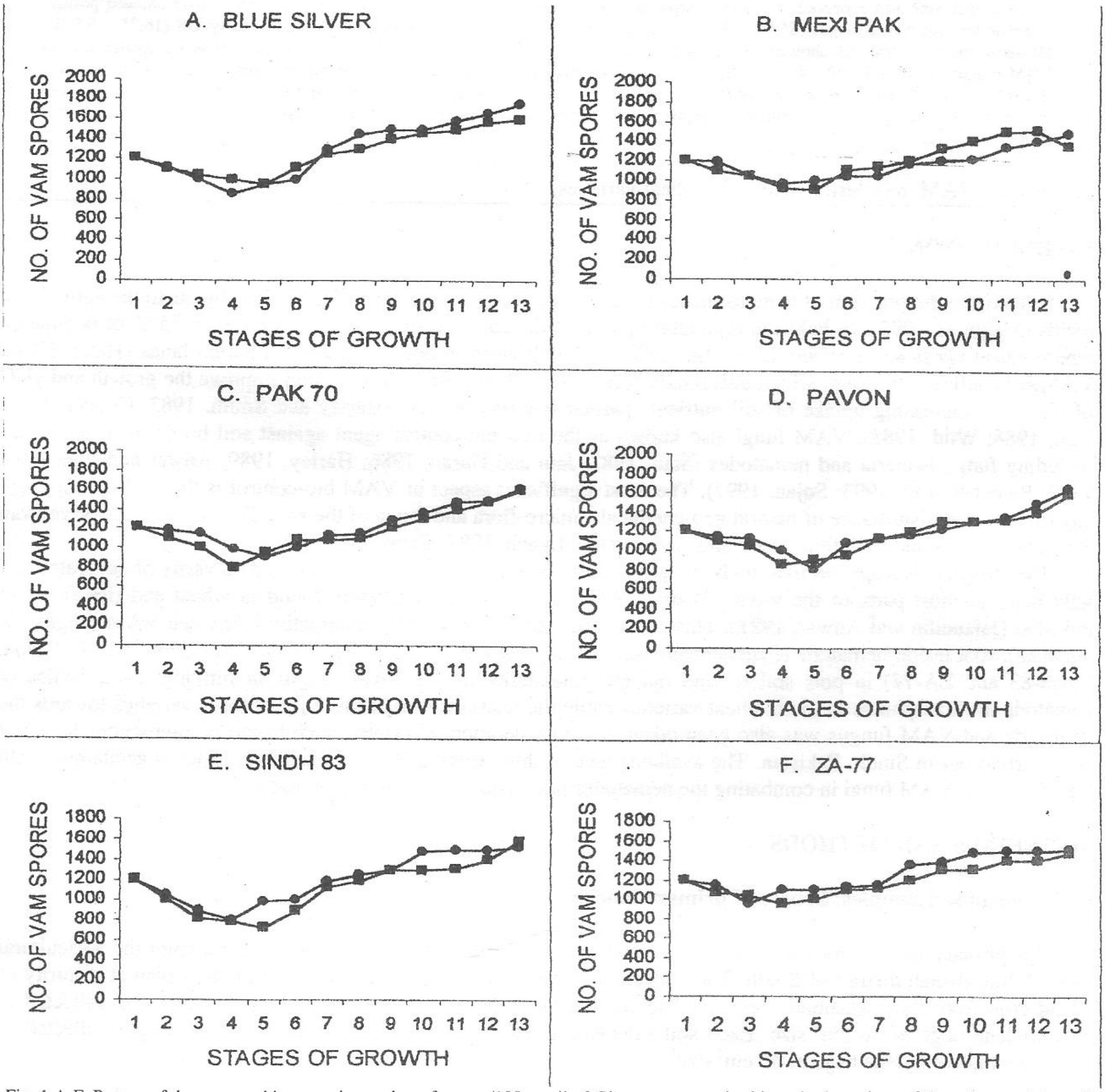

Fig. 1 A-F. Pattern of decrease and increase in number of spores/100 g soil of *Glomus mosseae* in rhizospheric regions of the wheat varieties with their progressing age during single and combined inoculation.

Study of VAM and nematode infections:

Ten days old wheat seedlings of the 6 wheat varieties were grown aseptically under lab condition on sterilized filter paper carefully transplanted into well washed and surface disinfected ($NaOCl_2$ 2 % v/v) baked clay pots @ 3 seedlings / pot (of 15 cm height with 12 and 8 cm top and bottom diams. respectively) containing 2 Kg steam sterilized (under 1.1 Kg /cm^2 at 121 C for 2 separate 1-hour period), sandy clay loam soil with true density 2.66 g /cc, pore space 43 % and pH level 7.2 supplemented with vermiculite @ 1.5 g / 100 g soil as growth medium. The soil was pre-inoculated with the inocula of *G. mosseae* @ 1200 spores /100 g soil and with *Pratylenchus* sp. @ 400 individuals /100 g soil. The population of both *G. mosseae* and *Pratylenchus* sp. was kept almost same as found in natural agricultural field soil of Sindh. In this way 3 replicate pots were made for each wheat var. The transplanted pots containing *G.mosseae* + *Pratylenchus* sp. were placed by completely randomized designed method in screen house under natural condition along with separate sets of control pots (*G. mosseae* / *Pratylenchus* sp.). The seedlings were then regularly watered with distilled water up to maturity of crops.

The root and soil samples of each var. of wheat were collected from the treated and control set of pots after 10 days intervals for comparative and detail studies of VAM and nematodes population / lesions with the roots of 6 wheat varieties VAM infection in root tissues was determined by the technique of Philips and Hayman (1970) modified by Koske and Gamma (1989) and VAM infection was assessed (quantified) by slide length method (Givonetti and Mosse, 1980) while the root lesion caused by the *Pratylenchus* sp. was determined in term of diseases severity (DS) given by Mayee and Datar (1986).

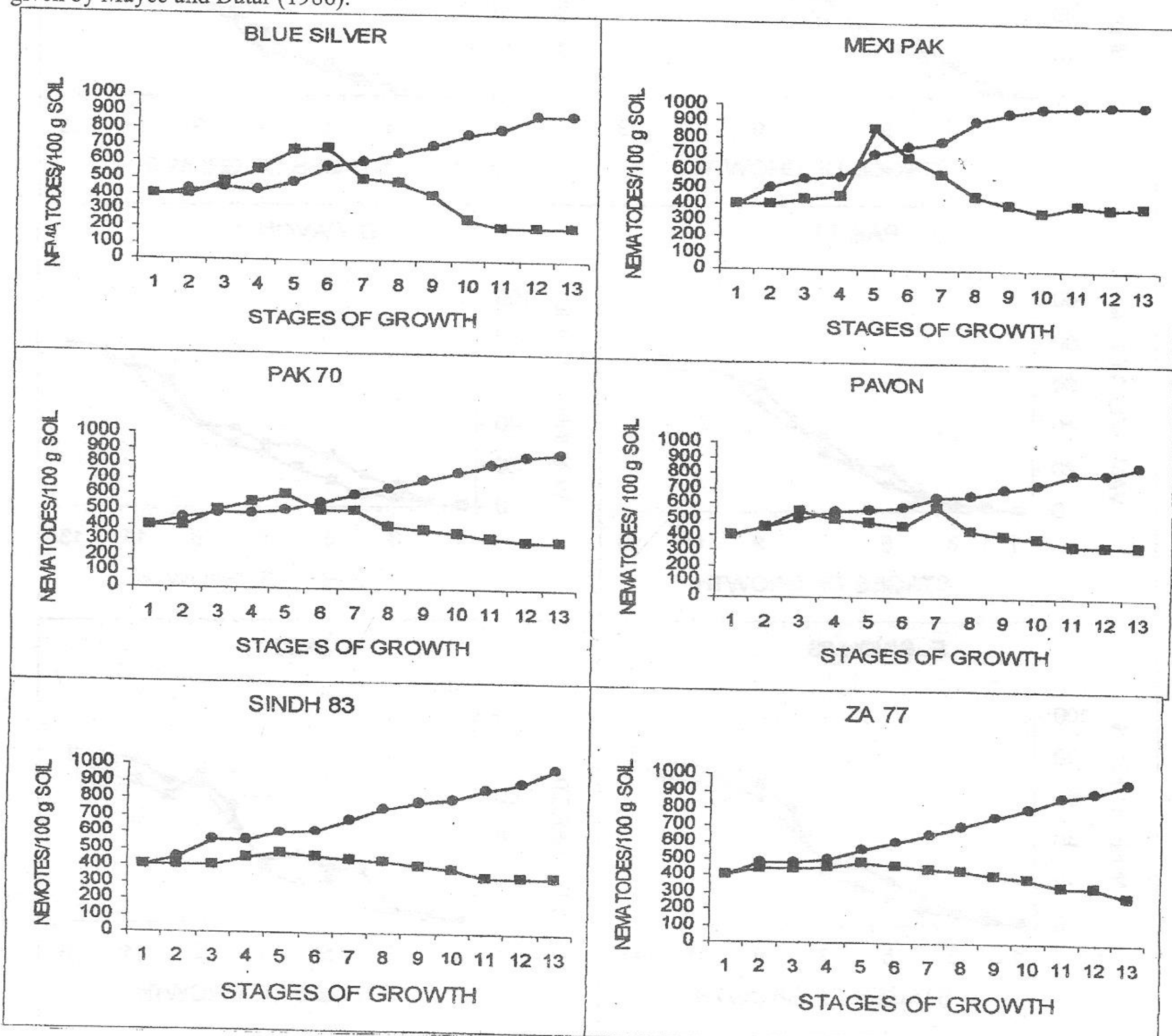

Fig. 2 A-F. The pattern population of *Pratylenchus* sp. /100 g soil in the rhizospheric regions of 6 wheat varieties with their progressing age during single and combined inoculation.

RESULTS

The spores (population) of *G. mosseae* in soil pots of all the 6 wheat varieties were reduced initially in both single and combined (*Pratylenchus* sp.+ *G.mosseae*) inoculated pots and then exponently increased up to the crop maturity (**Figs 1A–1F**). However, the pattern of increase and decrease was more or less same in both single and combined inoculated pots (**Figs. 1A–1F**). At crop maturity the highest number of VAM spores was observed in the rhizospheric region of var. Blue Silver (1760 and1610 spores / 100 g soil) while it was lowest in rhizospheric region of Mexi-Pak (1480 and 1360 spores/100 g soil) in single and combined inoculated pots respectively (**Figs. 1A and 1B**). The remaining 4 wheat varieties showed VAM spores population in between the two extremes (**Figs. 1 C, D, E and F**).

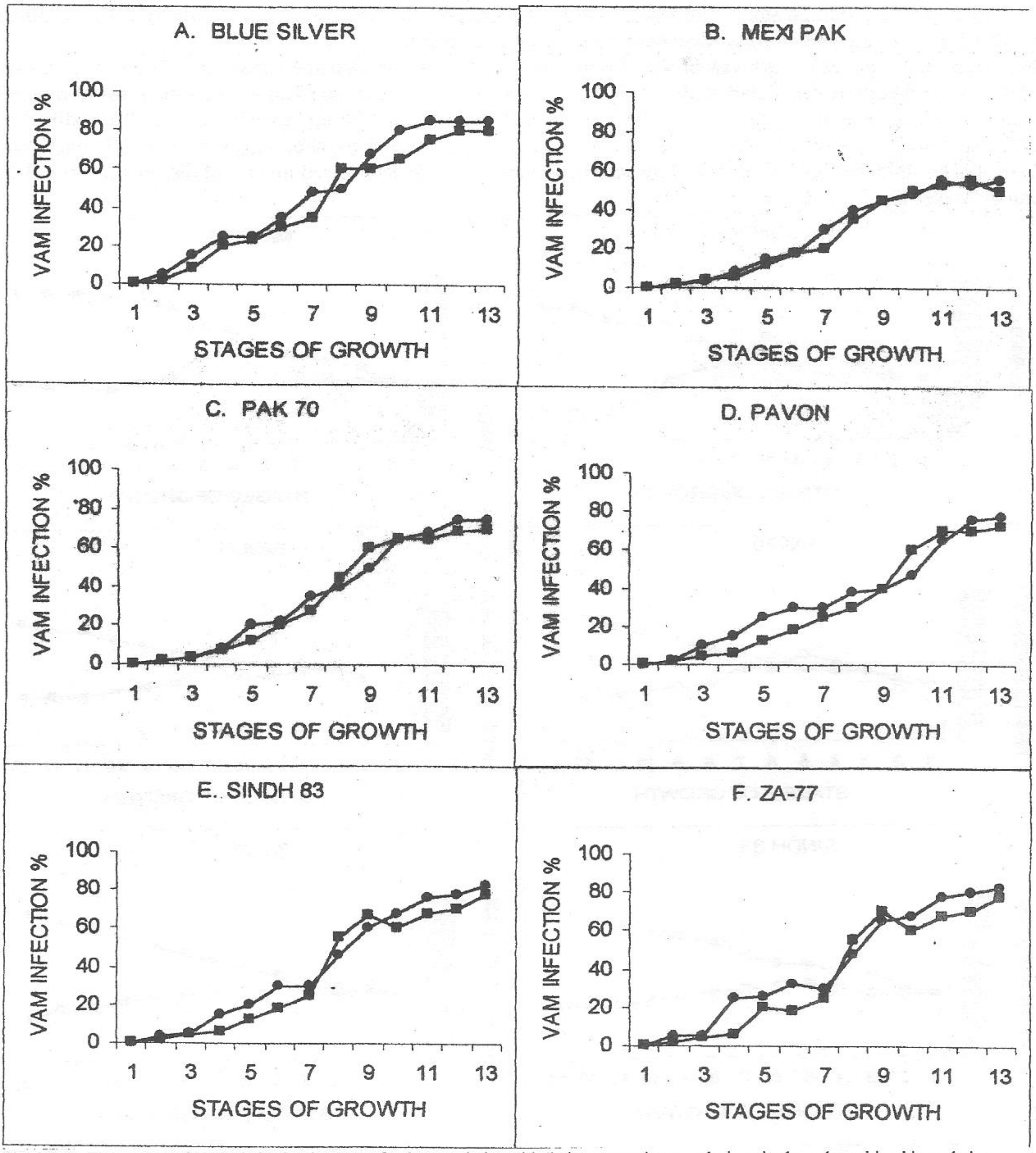

Fig. 3 A-F. The pattern of VAM infection in roots of wheat varieties with their progressing age during single and combined inoculation.

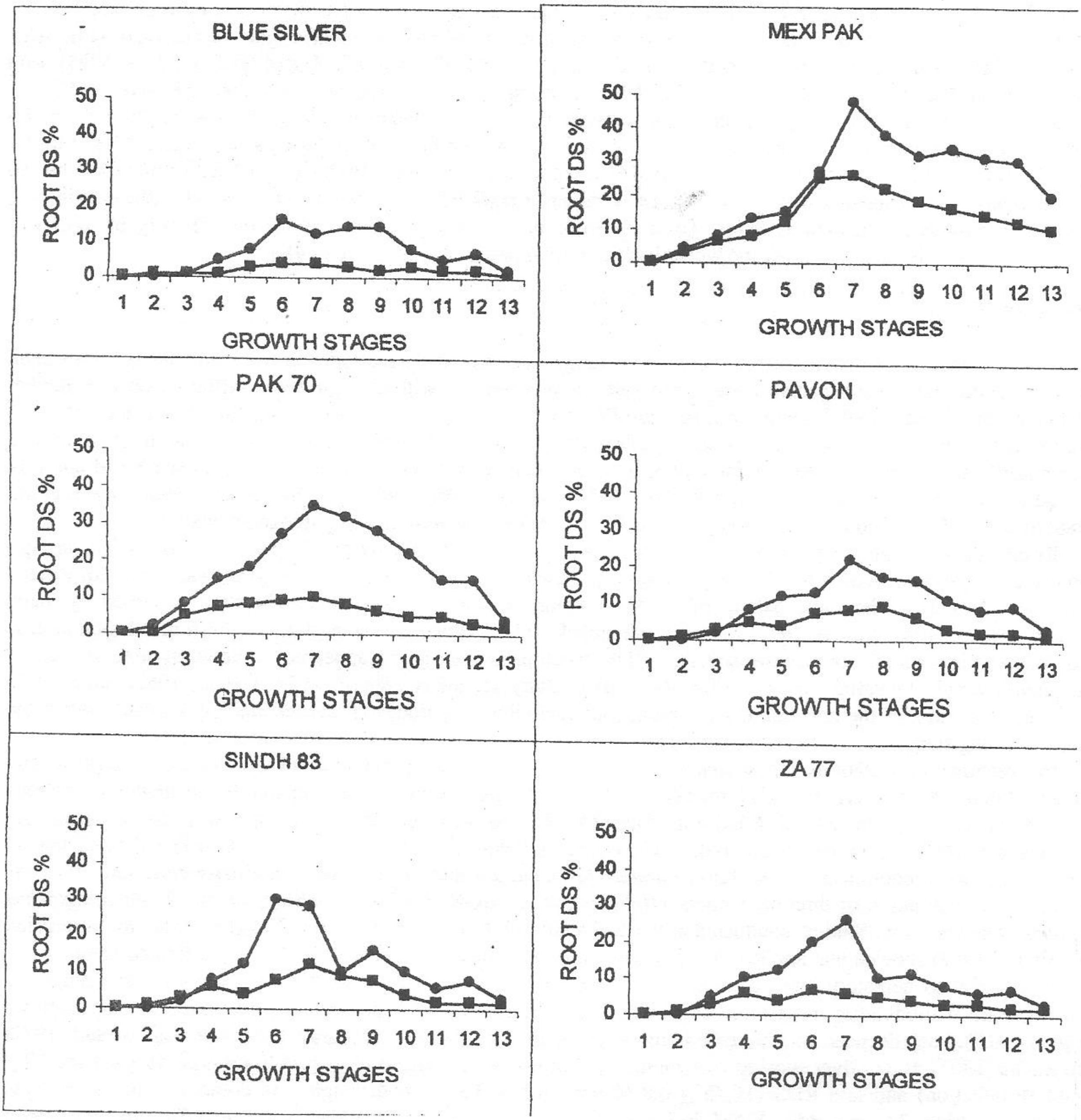

Fig. 4 A-F. The pattern of intensity of root lesions in term of root disease severity (RDS) caused by *Pratylenchus* sp. in the roots of 6 wheat varieties with their progressing age during single and combined inoculation.

In single inoculations the population of *Pratylenchus* sp. in pot rhizopsheric soil of all the 6 wheat varieties was found to be increased with the age of plants and reached highest at the crop maturity but differed variety wise (**Figs. 2A–2F**). The highest population of *Pratylenchus* sp. was observed in pot soil of wheat var. Mexi-Pak (1170 /100 g soil) and less in Blue Silver (850 / 100 g soil) (**Figs. 2 A and 2B**). However, the combined inoculations showed significant reduction ($P<0.05$) in the population of *Pratylenchus* sp. in rhizospheric regions of all wheat varieties except in Mexi Pak as compared to control set of pots (**Figs. 2A–2F**).

Roots of all the 6 wheat varieties in both single (*G. mosseae / Pratylenchus* sp.) and combined inoculated pots (*Pratylenchus* sp.+ *G.mosseae*) were found to be infected with VAM fungus after 10 days of transplantation then increased exponentially in sigmoid fashion (**Figs. 3A–3F**). The pattern of increase was almost same in both the pots with slight variations (**Figs. 3A–3F**). However, the var. Blue Silver showed more positive response towards *G. mosseae* therefore, highest VAM infection percentage was found in its root tissues at its maturity (85.58 $\pm$ 4.7 and

82 ± 07.44 % infection) (**Fig. 3A**), while it has shown least response to *Pratylenchus* sp. in term of root diseases severity–RDS (16.75 ± 6.8 % and 04.5 ± 3.8) in single and combined inoculated pots respectively (**Fig. 4A**). However, Mexi Pak showed susceptibility towards the nematode (48.08 ± 08 % and 25.5 ± 2.9 % RDS) with prominent root lesions and stunted growth (**Fig. 4B**) and comparatively less response towards VAM fungus (55.18 ± 7.7 and 49 ± 15 % infection) (**Fig. 3B**) in single and combined inoculations respectively as compared to the rest of 5 varieties (**Figs. 4A and 4B**). The rest of the 4 wheat varieties showed in between these two extremes (**Figs. 3 and 4 C, D, E and F**). The results also showed that VAM infection % progressed with the age of plants while RDS caused by *Pratylenchus* sp. appeared with high intensity in between 60–70 days old plants then naturally declined in both single and combined inoculations (**Figs. 4A–4F**). However, in combined inoculations the intensity of RDS was reduced significantly (P<0.05) in all the wheat varieties except Mexi Pak (**Figs. 4A– 4F**).

DISCUSSION

A wide range of parasitic soil organisms like fungi, bacteria, and nematodes derive their energy from plant roots. In nature, all these organisms may exist side by side with or without significant influence on one another (Giannakis and Sanders, 1990). However, the significant influence of VAM in nutrition uptake by the plants is well known all over the world (Abbott and Robson, 1981; Harley and Smith, 1983; Nicolson, 1967; Wild, 1988). Along with nutritional benefit of VAM several studies have also been carried out on interactions between VAM and root disease causing organisms (Schenck and Kellam, 1978; Jain and Sethi, 1988; Pinochet *et al.*, 1993; Umesh *et al.*, 1988; Sujan, 1997) to find out the efficacy of VAM in combating the activities of pathogenic nematodes.

Results on single and combined inoculations (*G. mosseae* / *Pratylenchus* sp.; *G. mosseae* + *Pratylenchus* sp.) showed the development of VAM infection in sigmoid fashion in root tissues of all the wheat varieties with varietal variations (**3A– 3F**). The same pattern of VAM infection in many crops was already been described by many workers (Mosse, 1981; Sutton, 1973; Land and Shonbeck, 1991). However, the high intensity of root lesion disease was observed in term of root diseases severity (RDS) when prominent lesion appeared (in between 60 to 70 days of old plants) which thereafter declined (**Fig. 4A – 4F**). Many scientists (Wiese, 1977; Dehne, 1982; Sujan,1997) described that most of the soil borne plant pathogens including nematodes (*Pratylenchus* spp.) usually infect the plants at young stage.

In combined inoculations (*G.mosseae* + *Pratylenchus* sp.) the population of the nematode significantly decreased in pot rhizospheric soil of all the six wheat varieties after initial rise as compared to the single inoculations of *Pratylenchus* sp. except the var. Mexi-Pak (**Figs. 2A–2F**). Our findings can be explained with the studies of Jain and Hasan (1986) carried out in the Indian Grassland and Fodder Research Institute (IGFRI) indicated that in presence of two predominant VAM fungi *Glomus fasciculatum* and *G. mosseae* in rhizosphere soil of forage sorghum; the population of three nematodes (*Helicotylenchus dihystera*, *Pratylenchus zeae* and *Tylenchorhynchus vulgaris*) was very low. Studies conducted at the University of Agriculture Sciences, Banglore India by Sitramaiah and Sikora (1982) showed that application of *G. fasciculatum* reduced nematode infection in the tomato plants.

In contrast to nematode the population of *G. mosseae* in pots soil of all the six varieties either single or combined inoculations after initial decrease, increased continuously and reached to its maximum at crop maturity (**Figs. 1A–1F**). Such decrease and increase in the VAM spores population corroborated with the result of Saif (1977) and Baylis (1967). Blue Silver showed maximum VAM infection percentage in its root tissues (85.58 ± 4.7 and 82 ± 07.44 % infection) and less RDS (16.75 ± 6.8 % and 04.5 ± 3.8) in both single and combined inoculated pots respectively (**Figs. 3A and 4A**). VAM infection was observed lowest in wheat var. Mexi-Pak and it showed susceptibility towards the nematode (48.08 ± 08 % and 25.5 ± 2.9 % RDS) (**Fig. 4A**) and comparatively less response towards VAM fungus (55.18 ± 7.7 and 49 ± 15 % infection) (**Fig. 3B**) in both single and combined inoculations respectively. It can be explained as different varietal responses of different wheat varieties which may be specifically resistant or susceptible towards VAM and nematodes (Vanderplank, 1984).

Combined inoculations showed reduction in both the population of nematode and RDS in roots of all the wheat varieties Dehne (1982) explained hat parasitization of plants root by nematode can be influenced by the establishment of VAM infections. He also explained that in general mycorrhizal plants suffer less damage and incidence of disease decreased or pathogen development is inhibited. The population nematodes decreased as soon the VAM infection established due to alteration in root exudates or morphological alterations (Dehne, 1982; Sujan, 1997). It supports our findings that the population of *Pratylenchus* sp. initially increased in pot soil of all wheat varieties but soon decreased as VAM infection established in their roots (**Figs. 2A – 2F and 4A – 4F**). It can be further supported by Hussay and Roncadori (1977) who found out the reduced number of *Pratylenchus brachyurus* in rhizosphere soil of cotton in the presence of VAM fungi.

In our experiments the rate of penetration of *Pratylenchus* sp. into the root tissues also found to be reduced (**Fig. 5**) with reduced development of the nematode inside the root tissues, therefore, the damages caused by the nematode were also found low. Our experimental results are in accordance to Dehne (1982). It has also been described that the damage done by plants parasitic nematodes is compensated by VAM and some time high production of spores and high colonization of VAM in presesnce of nematodes also reduce the damages (Lugham, 1988). Sujan (1997) explained that 480 spores of VAM per plant and root colonization level 87 % in *Gossypium hirsutum* plants greatly reduced the eggs and nematode densities in root tissues and in soil.

CONCLUSION

It is concluded that all the 6 wheat varieties showed diverse varietal responses towards VAM and *Pratylenchus* sp. The population of *Pratylenchus* sp. in rhizosphere and root lesions caused by it in roots of all the 6 wheat varieties except variety Mexi Pak was found to be reduced in presence of *G. mosseae* perhaps due to alteration in roots exudation or lignification. Blue Silver found as a nematode resistant wheat varieties.

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