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Effect of soil amendment on pupulation of *Aspergillus flavus,* the aflatoxin causing fungus, in chilli rhizosphere soil

S. SUDHA AND M. K. NAIK
Department of Plant Pathology, University of Agricultural Sciences, Raichur 584 102, Karnataka,
Email : manjunaik2000@yahoo.co.in

Soil serves as a reservoir for *Aspergillus flavus* which in turn becomes a primary inoculum for directly infecting fruits. neem cake and *Trichoderma* enriched farm yard manure were amended before planting. The *Aspergillus flavus* pupulation was enumerated from the amended soils at an interval of 30, 60, 90 and 120 days after transplanting. The population was not much variable in neem cake (490 cfu g^{-1} soil) and *T. viride* (479 cfu g^{-1} soil) amended plots as compared to control (507 cfu g^{-1} soil) amended plots as compared to control (507 cfu g^{-1} soil) at 30 days. But the population slightly increased at 60 days in both the amended plots. However, a significant reduction in pupulation was seen at 90 and 120 days after planting. The population was 695 and 365 cfu g^{-1} soil in neem cake applied plots and 434 and 237 cfu g^{-1} soil in *T. viride* amended plots at 90 and 120 days as against control with 1313, and 1070 cfu g^{-1} soil that indicated as reduction in pupulation to the extent of 47.07 and 65.88 at 90 days and 66.94 and 77.85 per cent at 120 days after application of neem cake and *T. viride* plots respectively. Any reduction in pupulation at red ripening stage of chilli crop can bring down the chances of aflatoxin contamination. The present investigation on soil amendment derives its strength for recommendation to farming community based on its ability to reduce the primary inoculum in the soil.

Key words: Amendment, *Aspergillus flavus,* neem cake, *Trichoderma viride,* soil pupulation

INTRODUCTION

Chilli (*Capsicum annuum L.*) is an important spice and vegetable crop used all over the world in one form or the others. The global chilli area accounts for 1.5 million ha with a production around seven million tons. The largest producer of chillies in the world is India, accounting for 12-14 lakh tonnes of production annually followed by China, with a production of around four lakh tons. Mexico and Pakistan with around three lakh tons each. The major chilli producing states in India are Andhra Pradesh, Karnataka, Maharashtra, Orissa, Rajasthan and Tamil Nadu which contribute to 86 per cent of total area of chilli cultivation in the country and 90 per cent of the total Indian produce. India has exported 1.69 lakh tons of chilli in April-February 2007-2008 as against 1.23 lakh tons in the same period of 2006-07. The value of the export of chillies has been Rs. 906.44 crores as against Rs. 688.44 crores in the previous year (Anonymous, 2008).

Aflatoxin contamination and pesticide residues are the twin problems faced by Indian chillies in the global trade. Aflatoxins are poisonous secondary fungal metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* and a few related fungal species. They are potent carcinogenic, mutagenic and immuno-suppressive agents. Aflatoxin contamination has posed serious problems in commerce and international trade because of stringent quality standards imposed on aflatoxin contamination by many importing countries. According to European Union's Rapid Alert System for Foods and Feed (RASFF), three consignments of chilli powder have been rejected by the United Kingdom on July 5, 13 and 31 for the presence of aflatoxin above permissible limits. The presence of aflatoxin in the consignments

reportedly has been at 6.5 ppb (Potty, 2006). Aflatoxigenic fungi are common components of soil mycobiota. The infection occurs on stored fruits and the contamination with aflatoxin deteriorates quality and make the produce unfit for consumption, thereby hitting the export trade in the international market. The Northern Karnataka region is a potential for chilli cultivation in the irrigated black cotton soil due to the Tungabhadra and Upper Krishna project areas. However, aflatoxin contamination has been reported from this region on chilli (Ajith Kumar and Naik, 2005; Naik and Sudha, 2008). Soil serves as a reservoir for *Aspergillus flavus* which in turn becomes a primary inoculum for directly infecting fruits. Aflatoxigenic fungi reside in soil as conidia, sclerotia and hyphae which act as primary inoculum for directly infecting crops and also invading developing seeds of crops. Therefore, an understanding of the activities and population structure of aflatoxigenic fungi in soil is a pre-requisite for developing effective measures in managing aflatoxin contamination. Any management approach should aim at reducing the pupulation of *Aspergillus flavus* in the rhizosphere soil. The intervention to reduce aflatoxin contamination by foliar sprays can end up only in partial success since such practice will not reduce the primary load of inculum present in the soil. Hence, this paper directs towards interfering with the primary source of inoculum of *Aspergillus flavus* by amending the soil with neem cake and *Trichoderma,* the two eco-friendly amendments. In addition, the above amendments are considered to avoid the pesticidal residue problems on chilli likely to arise by the use of pesticides in mitigating aflatoxin contamination.

MATERIALS AND METHODS

A field experiment was conducted during *kharif* 2007 at Horticulture Garden, College of Agriculture, Raichur in order to reduce the soil population of *Aspergillus flavus* by amending the soil by neem cake and *Trichoderma viride.* The experiment was conducted in a plot measuring 42.5 m × 12.5 m with spacing of 75 × 45 cm in randomized block design (RBD). All other cultural and pest management practices were imposed as recommended in package of practices. The cultivar *Byadagi Kaddi* was used for transplanted in the month of August

Soil amendment

Neem cake

Neem cake was applied @ 250 kg/ha and was incorporated into the plots 10 days before planting

Amendment with FYM enriched with *Trichoderma*

Trichoderma viride was added @ 25 kg/ha to 2.5 t/ ha of farm yard manure (FYM) and sprinkling with water and were allowed to grow under the shade of a tree for 10 days. The FYM thus enriched was incorporated into the plots 10 days before planting.

Enumeration of A. flavus from amended soil

The amended soils at different intervals at 30, 60, 90 and 120 days after transplating was recorded by enumerating on selective media. The soil samples were air-dired, ground and filtered through muslin cloth. The population of *A. flavus* was enumerated using Czapeck's media. One g of soil was taken in 9 ml water, and serially diluted up to the concentration of 10^{-3}. One ml 10^{-3} of soil suspension was taken and pour plate method was used for enumeration. The colonies of *Aspergillus flavus* appearing after four days of incubation were noted. Number of colony forming units cfu g^{-1} of soil was counted and expressed as per g of soil

RESULTS AND DISCUSSION

After application of neem cake and *T. viride* in soil, the population of *A. flavus* was assessed at 30, 60, 90 and 120 days after application. The population was not much variable in neem cake (490 cfu g^{-1} soil) and *T. viride* (479 cfu g^{-1} soil) amended plots as compared to control(507 cfu g^{-1} soil) at 30 days after amendment with only 3.35 and 5.52 per cent decrease in population of *A. flavus* respectively. In other words, the population of *A. flavus* decreased marginally in the neem cake and *T. viride* amended soils respectively till 30 days after planting. But the population slightly increased at 60 days both in neem cake (792 cfu g^{-1} soils) and *T. viride* (606 cfu g^{-1} soil) amended plots. However, there was a significant reduction in population at 90 and 120 days after planting. The population was 695 and 365 cfu g^{-1} soil at 90 and 120 days respectively that

clearly indicated a reduction in population of *A. flavus* to the extent of 47.07 and 65.88 at 90 days and 66.94 and 77.85 per cent at 120 days after application of neem cake and *T. viride* plots respectively (Table 1). The incidence of *Aspergillus flavus* was 2.16 per cent in *Trichoderma* amended soil and 2.60 per cent in neem cake amended soil as against control with 7.41 per cent in the present investigation. The aflatoxin contamination had generally been noticed at the time of harvesting when the fruits were at red ripening stage or beyond that stage. The population of *A. flavus* was known to be the dominant factor in determining the serverity of aflatoxin contamination, in addition to other factors such as soil and climatic conditions and the cultivar planted. In this context, reduction of population at 120 days had a practical significance as it just coincided with red ripening stage of chilli fruit. The extent of aflatoxin contamination was directly co-related with that of soil population of *A. flavus* in different geographical regions of Northern Karnataka (Naik and Sudha 2008). The same had also been proved in case of groundnut aflatoxin contamination at USA (Bruce, 2006). Any reduction at red ripening

Table 1 : Effect of neem cake and *Trichoderma viride* on *Aspergillus flavous* population in chilli rhizosphere

Days/ Intervals	Treatment	Pupulation of *A. flavus* (cfu/g of soil)	Per cent decrease over control	Per cent change over base pupulation
	Neem cake	490	3.35	–3.35
30	*Trichoderma viride*	479	5.52	–5.52
	Control	507	—	—
	Neem cake	792	31.13	35.98
60	*Trichoderma viride*	606	47.30	16.33
	Control	1150	—	55.91
	Neem cake	695	47.07	27.05
90	*Trichoderma viride*	434	66.94	–17.28
	Control	1313	—	61.38
	Neem cake	365	65.88	–38.90
120	*Trichoderma viride*	237	77.85	–113.92
	Control	1070	—	52.61

stage of chilli crop could bring down the chances of aflatoxin contamination severity. At that stage if there was no amendment or incorportion either with *Trichoderma* or neem cake perhaps the population would had reached the level of 1070 cfu g^{-1} soil with subsequent increase would had resulted in severity of aflatoxin contamination. The previous results indicated that there was a decrease in population of *A. flavus* in soil samples from the plots applied with *Trichoderma* sp. (T-28) compared to control (Thakur *et al.*, 2003). The similar results were also obtained by Wani (2004) by use of neem products. The present investigation on soil amendment with neem cake and *T. viride* derives its strength for re-commendation to farming community based on its ability to reduce the primary inoculum present in the soil there by giving way for less aflatoxin-contamination.

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