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BIOCONTROL AND ALLELOPATHIC EFFECTS OF *EUCALYPTUS CAMALDULENSIS* DEHNH. LEAF LITTER ON THE GROWTH OF GREEN GRAM *(VIGNA RADIATA* L.) WITH FARMYARD MANURE

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ABSTRACT

The influence of Farm Yard Manure (FYM) was studied by examining seedling growth and some important physiological and biological aspects in green gram *(Vigna radiata* (L.) Wilczek), against *Eucalyptus camadulensis* Dehnh. leaflitter. FYM was applied as soil amendment to check their effects alone or in combination with litter on green gram growth. The soil amendment with *E. camaldulensis* leaflitter @1%(w/w) and 5%(w/w) along with FYM significantly reduced the inhibitory effects of *E.camaldulensis* leaf litter treatments and increased germination, shoot and root length, shoot fresh and dry weight, root fresh and dry weight of *Vigna radiata* L, while only leaf litter treatment showed suppression in above growth parameters. Both concentrations of leaf litter (1%w/w and 5%w/w) considerably decreased chlorophyll and protein content. The inhibitory effect of leaf litter at high concentration on total protein and soluble carbohydrate was significantly controlled by FYM amendment. Free proline and nitrate reductase activity increased with increasing litter concentration. The treatment of Eucalyptus leaf litter extract also enhanced the accumulation of phosphorus in green gram.

Keywords: Allelopathy *Eucalyptus camaldulensis*, Litter, chlorophyll, protein content, green gram, *Vigna radiata*.

INTRODUCTION

Agroforestry is a form of integrated land use systems which involves the combination of woody perennial plant with annual crops to derive various benefits and utilities from the same piece of land however, the mixed crops tend to be incompatible and interfere with one another above and below the ground. The interference is mainly due to harmful effects of plant competition (for nutrient, water and light) and allelopathy. In Pakistan, Eucalyptus trees have been established to be an integral part of our agroforesty system, because of its fast growing nature. *Eucalyptus* species including *E. grandis, E. camaldulensis, E. tereticornis, E. globules, E.urophylla, E. viminalis, E.saligna, E.deglupta, E.exserta, E.citriodora and E.robusta.* Eucalyptus trees are extensively grown in canal commended areas for reducing water table and salinity. The eucalypts species are considered to be as of the most notorious of allelopathic trees causing understory suppression specially in dry climates (rainfall< 400mm) (May and Ash, 1990). Debris from the tree pruning and litter reduces agricultural yields, whereas the leaves are unpalatable and allelopathic to pasture growth. Several studies (Alexander, 1989; Kohli *et al*., 1988) revealed that large areas of the ground beneath the *Eucalyptus* remains completely bare and ground vegetation is very limited in extent. Khan *et al*. (2004) reported that *Prosopis*, *Eucalyptus* and *Acacia* retarded the growth and development of several weeds. Therefore, *Eucalyptus*, though a potential industrial crop is not being recommended as an inter crop in an agro-foresty system (Bansal, 1988; Suresh and Rai,1987), presumably due to the release of allelochemicals and phytochemical compounds from the tree (Lisanework and Michelson, 1993). Mukhopadhya *et al.* (1995) reported that extracts of eucalyptus decreased the plant growth of winter crops and they concluded that the inhibitory effects of eucalyptus leaf extract on germination and growth was attributable to its essential oil contents. Similar findings have been reported by other workers (Blaise *et al.*, 1997; Thaukar and Bhardwaj, 1992).

Iqbal *et al.* (2003) found 16 components in the essential oil of *E.camaldulensis* out of which 5 compounds (alpha pinene, 3-carene, beta-phellandrene, 1-8 cineole and p-cymene) were identified. Ghafar *et al*. (2000) found that these allelochemicals and volatile compounds present in all parts of *E.camaldulensis* have harmful effect on the crops in the ecosystem resulting in the reduction and delaying of germination mortality of seedling and reduction in growth and yield. Vaughan and Ord (1990) and Putnum (1984) reported that eucalyptus species released volatile compounds such as benzoic, cinamic and phenolic acids which inhibit growth of crops and also reduce the soil pH. The release of phenolic compounds adversely affected plant growth through their interference with energy metabolism, cell division, mineral uptake and other biosynthesis processes (Rice, 1984). Different researchers found that the eucalyptus leachate had varying degree of inhibitory and stimulatory effects on germination percentage (Phlomina and Srivasuki, 1996) and plant growth (Sidhu and Hans, 1998) and considerably at higher concentration (Jayakumar et al 1990). *Eucalyptus* tree belt had more adverse effect on wheat than legume crop. Inouye *et al*. (2001), Dawar *et al*. (2007) have reported that eucalyptus essential oil is considered to have marked antiseptic action against infectious bacteria, and fungi which helped in early growth parameters like increase in shoot, root length and shoot, root weight.

Eucalyptus tree is a fast producer of biomass because of the large amount of water and nutrient uptake as compared to many local plants and trees but very little return of humus to the soil because of slow decomposition of leaves. There is a rapid loss of nutrient reserves from the soil due to short rotation cropping of eucalyptus tree, therefore to maintain soil fertility and enhance their productivity, the use of other alternative option of soil fertility replenishment is indispensable. FYM is the potential source of nutrients. Several studies reported the efficiency and effectiveness of FYM as an organic nutrient source in maintaining soil fertility, improving crop yield and sustaining productivity. It is suggested that, in case of agroforestry, the allelopathic inhibitory effect of *E.camaldulensis* can probably be suppressed by organic fertilizer (i.e. farm yard manure). This study was initiated to determine the allelopathic effect of *E. camaldulensis* on the growth of green gram and suppression of allelopathic effect by the addition of farmyard manure.

MATERIALS AND METHOD

Sampling of leaf litter and farm yard manure: Naturally decomposing *E.camaldulensis* leaf litter was collected from a garden surrounded by rows of *E.camaldulensis* trees. The FYM was well decomposed under shade.

Experimental Design (Pot Culture): The experiments was conducted to determine the allelopathic effect of *E. camaldulensis* leaf litter on green gram growing under different concentration of litter leachate and FYM. In this experiment, the crushed *Eucalyptus* leaf litter and farm yard manure amended with soil at the ratio of 1:100 and 5:100 (w/w) in pots. Each pot contained 1 kg of soil treated with either 10 or 50gm of *Eucalyptus* leaf litter or FYM to achieve a ratio (w/w) of 1 % and 5 %. Mixture of the two was obtained by mixing leaf litter and farm yard manure equall amounts. Complete randomized design having three replicate for each treatment was used. Ten healthy imbibed seeds of green gram were planted in each pot together. The plants were irrigated at every two days with distilled water. The experiment continued for fifteen days, after which the plants were harvested and the final measurements were recorded for shoot length, root length, shoot fresh weight and root fresh weight. The dry weight of shoot and root were obtained after oven drying at 65C for 48 hours. The harvested crop was subjected to the analysis of physiological and biochemical test to check the allelopathic effect of eucalyptus leaf litter on some essential processes of plant, Chlorophyll estimation and carbohydrates were determined by the method of Arnon (1949) and Yemm and Willis (1956), respectively. Due to the presence of phenolic compounds in the treatment, Protein was determined by Bradford (1976) method. Proline was determined by Bates (1973) method, Nitrate reductase by Borden (1984) and Phosphorus concentration was determined by method described by Allen *et al*. (1974).

The percentage of inhibitory effect on shoot and root growth (length, fresh weight and dry weight) in comparison to control was calculated by Surendra and Pota (1978) formula. $I = \frac{100 - T}{C} \times 100$

Where, I is the parentage of inhibition t is treatment reading and c is control plant reading.

The data were analysed statistically

RESULTS AND DISCUSION

Table 1 & 2 showed that, the effect of *Eucalyptus* litter was totally inhibitory when supplied as soil amendment; this was probably due to natural decomposition of leaf litter inside the soil that made it more toxic for growth of plants. This result is in agreement with those reported by Smith (1989). He reported that some tree extracts negatively affect only seed germination while other affects plant growth. Mukhopadhyay *et al.,* (1995) reported that extracts of *Eucalyptus* decreased the plant growth of rabi crops and concluded that the inhibitory effect of *Eucalyptus* leaf extracts on germination and growth was attributed to the essential oil content.

Shoot length: Soil amendment with *E.camaldulensis* leaf litter had inhibitory effect on shoot length at both rate (1%w/w and 5%w/w) in gram. These inhibitory effects were efficiently reduced when leaf litter supplied with FYM. The inhibitory percentage decreased at combine treatment (L+FYM) from 33.29% to 2.58% at 1% (w/w) and 41.70% to 33.66% at 5% (w/w).

Root length: Table 3 showed that, root length of gram was inhibited by leaf litter application at both concentrations. Soil amendment with farm yard manure as combine treatment with litter was beneficial and results reduced eucalyptus leaf litter inhibitory effect from 58.46% to 34.46% at 1% (w/w) and 88.20% to 65.07% at 5% (w/w).

Shoot biomass: Table-3 showed that, the application of leaf litter has negative effect on gram shoot biomass. The litter treatment greatly reduced the shoot biomass from 51% to 55% in shoot fresh weight and 0.89% to 67% in shoot dry weight with increasing conc. However, the application of leaf litter along with FYM at low concentration (1% w/w) has positive effect on gram shoot biomass i.e. 41.78% increase in shoot fresh weight and 16.14% in shoot dry weight.

Table 1. Effect of *Eucalyptus camaldulensis* leaf litter and farm yard manure on growth of green gram (*Vigna radiata* L.).

Treatment		Shoot length (cm)	Root length (cm)	Shoot Fr. Wt. (g)	Root Fr. Wt (g)	Shoot Dry Wt. (g)	Root Dry Wt. (g)
T0	Control (no leaf litter no FYM)	18.2 a (-)	14.83 a (-)	2.13 d (-)	0.33 b (-)	0.223 d (-)	0.063 (-)
T1	1% Leaf litter	12.14 e (-33.29)	6.16 d (-58.46)	1.04 e (-51.17)	0.5 c (+51.51)	0.221 e (-0.896)	0.143 (+126.98)
T2	1% FYM	17.88 b (-1.76)	11.06 b (-25.42)	3.55 a (+66.66)	0.29 e (-12.12)	0.479 a (+114.79)	0.077 (+22.2)
T3	1% Litter+1% FYM	17.73 c (-2.58)	9.72 c (-34.46)	3.02 c (+41.78)	0.49 a (+48.48)	0.259 c (+16.14)	0.155 (+146.0)
T4	5% Leaf Litter	10.61 g (-41.70)	1.75 g (-88.20)	0.95 f (-55.40)	0.32 d (-3.03)	0.073 g (-67.26)	0.034 (-46.03)
T5	5% FYM	16.44 d (-9.67)	3.76 f (-74.64)	3.43 b (+61.03)	0.21 f (-36.36)	0.352 b (+57.84)	0.122 (+93.65)
T6	5% Litter+5% FYM	11.71 f (-33.66)	5.18 e (-65.07)	0.95 g (-55.40)	0.13 g (-60.60)	0.211 f (-5.38)	0.116 (+84.126)

*Values in parenthesis indicate percent increase (+) or decrease (-) over control.
Different letters indicates a significant difference at 0.05 level of probability ($p<0.05$) according to DMRT

Root biomass: Treatment of leaf litter single or along with FYM showed the increase in root fresh and dry weight of gram. High rate of biomass production appear at 1 % (w/w) litter and 1 % (w/w) mixture (L+FYM). Table3 showed that gram root dry weight increased with L+FYM treatment i.e. 84.126% at 5%w/w and 146.0% at 1%w/w. This increase in root biomass indicates an environmental stress due to the presence of allelochemicals. Einhelling and Leather (1988) declared that the storage of excess biomass in the root might be considered as conservative strategy to manage allelopathy. Khan *et al.* (2007) also found the effect of aqueous extract of *Eucalyptus* stimulatory on root and shoot of maize seedlings.

Table 2. Effect of Eucalyptus leaf litter on chlorophyll content of Green gram.

Treatment		Chl "a" mg/g fr.wt	Chl "b" mg/g fr.wt	Total Chl mg/g fr.wt
T0	Control (no leaf litter no FYM)	1.894 (0.0)	1.067 (0.0)	2.961 (0.0)
T1	1% Leaf litter	0.821 (-56.65)	0.596 (-44.14)	1.42 (-52.04)
T2	1% Fym	1.055 (-44.30)	0.665 (-37.68)	1.72 (-41.91)
T3	1% L+ 1% FYM	1.063 (-43.88)	0.775 (-27.37)	1.84 (-37.86)
T4	5% Leaf litter	0.703 (-62.88)	0.738 (-30.83)	1.44 (-51.37)
T5	5% Fym	0.595 (-68.58)	0.394 (-63.07)	0.99 (-66.57)
T6	5% L+ 5% FYM	0.424 (-77.61)	0.387 (-63.73)	0.811 (-72.61)

DISCUSSION

Many phenols have catechol groups and at high conc. (i.e. 5% in the present study) have the ability to chelate divalent or trivalent metal ions (Crawley, 1997) and inhibit ion uptake. Puri and Khara (1991) also obtained the same effects of *eucalyptus teriticornis* on *phaseolus vulgaris*. The uptake of NPK was highly correlated (p>0.01) with dry matter production which may indicate that as the nutrient uptake decreased total dry matter showed a corresponding decreased. Similar result was reported by Taiz and Zeiger(1998).

Table 2 showed that Chl.a and Chl.b of gram was significantly decrease in litter treatments mainly due to low uptake of N and other nutrients, this decreased in chlorophyll content in litter treatment possibly caused by the interactions of P with Fe uptake in growth medium. It is well known fact that iron containing enzyme is needed in chlorophyll formation. (Alam, 2001; Konar and Kushari, 1995) found chlorophyll content reduction in *costus speciousus* and finger millet.

Effect of *E. camaldulensis* leaf litter on plant metabolic products:

Biotic and a biotic stresses during growth often cause plants to increase the production of metabolic compounds i.e. free amino acids, proline, sugar and other organic solutes. Green gram also tends to accumulate all these above, which may be considered as an adaptive mechanism to increase stress tolerance (El-Darier and Youssef 1998). High content of carbohydrate along with high application of additional solutes i.e. litter and FYM (alone or combine) showed the conservative adaptability in order to increase biomass toward external stress.

Table 3: Influence of E. *camaldulensis* litter and farmyard manure on soluble carbohydrate, Protein, nitrate reductase and free proline contents of green gram.

Treatments	Soluble Carbohydrate	Total Protein	Nitrate Reductase	Free Proline
	µmole/gm fr.wt	µmole/gm fr.wt	µmole/gm fr.wt	µmole/gm fr.wt
Control	15.95	7.9	0.023	13.2
1% Leaf litter	11.48	7.8	0.033	9.43
1% FYM	9.66	7.77	0.023	9.5
1% L+ 1%FYM	10.96	7.67	0.038	8.37
5% Leaf litter	9.05	6.42	0.04	9.54
5% FYM	13.48	8.17	0.04	8.99
5% L+ 5%FYM	14.54	7.09	0.037	8.88

Eucalyptus leaf litter application reduced Carbohydrate content in both plants as compare to control. In Green gram: Inhibitory effect of high litter application on soluble carbohydrate content was significantly reduced in combine application of litter with farm yard manure. There are two factors which enhanced the carbohydrate production at 5% L+FYM **.** First is the conservative strategy of plant to face external stress condition, Second is the presence of farm yard manure that reduced litter inhibitory effect and support carbohydrate production.

In green gram, Phenolic compounds of litter increase low molecular wt peptide synthesis in plant. Thus increase total protein content. Although at high concentration litter supply reduced protein content but the combined treatment (5%L+FYM) reduced the inhibitory effect of high litter supply.

Ugrekhelidze *et al*. (1990) discussed the transformation of phenol ($14C_6H_5OH$) penetrating through the roots of mung been sterile seedlings. Phenol was coupled to low molecular weight peptide producing phenol-peptide conjugates. Hydrolytic cleavage of the conjugates liberated initial labeled phenol and some unlabeled amino acids. It was suggested that the conjugation is carried out via the hydroxyl group of phenol and functional groups of peptides .conjugation with low molecular wt. peptides is considered to be the main path way for phenol detoxification, since about 60%of phenol absorbed by plants conjugates with peptides. In the plants treated with phenols, the amount of low molecular wt. peptides is increased. The increased in peptides synthesis in plant seems to be induced by the penetration of toxic phenol molecules in the cell. The small amount of phenol molecules assimilated through is transformed via aromatic ring cleavage and dibasic carbonic acid formation. Different phenolic compounds: Salicylic acid (monophenol), resorcinol (diphenol) and tannic acid (poly phenol), when applied exogenously on mung plant at seedling and flower initiation stages (15 and 65 days after sowing) enhanced free amino acid and soluble proteins.

In green gram litter application enhanced free proline amount in both crops. Biotic and abiotic stresses during growth often cause plants to increase the production of metabolic compounds i.e. free amino acids, proline, sugar and other organic solutes .

Total phosphorus:

Table 4. Phosphorous content in green gram under various treatments.

Total Phosphorus			
Sample		µmole/g fr.wt	% = PPm x R/WT x 100/10000
T0	CONTROL	2.72	0.02
T1	1% LEAF LITTER	81.87	0.5
T2	1% FYM	8.19	0.06
T3	1% L+1% FYM	10.91	0.08
T4	5% LEAF LITTER	54.58	0.03
T5	5% FYM	2.73	0.02
T6	5% L+5% FYM	116.8	0.7

Phosphorus content was low in water applied plant (Table 4). The other probability reason could be an easy leaching out of available phosphorus in water application as compared to the leaf litter application, where possibly the allelochemical compounds hindered its released and leaching. Similar finding has been reported by Khitran (1996).The treatment of Eucalyptus leaf litter enhanced the accumulation of P in green gram.

Conclusion: In the present study, green gram growth parameters such as shoot and root length, shoot and root fresh weight, shoot and root dry weight were markedly affected by the application of *E.camaldulensis* leaf litter amended with soil, due to microbial decomposition of allele-chemicals of litter extract making it toxic for plant growth. The phenolic compounds released from the decomposition of E. *camaldulensis* leaf litter due to its allelopathic interference caused an inhibition of a number of interacting physiological processes which were the main factor which suppressed growth rate. The interference of nutrient accumulation is one of the most effective mechanisms of phenolic compound action. Several volatile and water-soluble toxins were found in Eucalyptus litter. These adsorbed terpenes were toxic to germinating seeds and seedlings, and inhibited plant growth. The study indicated that the phyto-toxicity of phenolic compounds was present in *E. camaldulensis* leaf litter as allele-chemical was highly significant but could be overcome by the application of farm yard manure as bio-control agent.

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