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Metabolic Changes in Broad Bean Infected by *Botrytis fabae* in Response to Mushroom Spent Straw

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Abstract: Spent wheat straw (after cultivation of oyster mushroom, *Pleurotus floridanus*) was infested into the soil at different concentrations (1, 2, 5% w/w) to study its effect on the growth of broad bean (*Vicia faba*) susceptible to *Botrytis fabae*. In addition, the effect of spraying the plant with its water extract was also studied. In seedling stage, the non infected broad bean plants treated with 1 and 2% spent straw (ss) showed an increase in shoot length and at 5% ss as well as upon spraying with water extract of spent straw (wess) root length increased. All treatments increased both root and shoot length in infected plants only, while the number of leaves was increased in both infected and non infected plants. The number of nodules and lateral roots, fresh and dry weight of both shoot and root increased at 2 and 5% ss. Moreover, spraying with wess increased fresh and dry weight of root in all plants. In non infected plants, treatment with wess and 2% ss increased glucose, sucrose and total carbohydrate in both root and shoot, in addition to increasing sucrose at 1%. On the other hand, glucose contents were significantly increased at 5% ss while all treatments increased sucrose in root and at 1% in shoot of infected plants. Also, increasing in polysaccharides in whole plants occurred at 2 and 5% as well as with wess. However, all treatments increased total carbohydrates in root of the infected plant. Total nitrogen was significantly increased only in infected plants when sprayed with wess and treated with 5% ss. In flowering stage, all treatments increased shoot length in non infected plants. However, 2 and 5% ss increased both root and shoot length of the infected plants. Increasing the number of rootlets and nodules in the non infected plants was recorded at 2% ss but, in infected ones an increase was apparent at 2 and 5% ss. Increase in fresh and dry weight of root was achieved at 1 and 2% ss in non infected plants, and at 2 and 5% ss in infected ones. Shoot fresh and dry weight increased at 2% in all plants. Glucose content of whole non infected plants was significantly increased at 1 and 2% ss. However, 5% ss and (wess) increased glucose content in both root and shoot respectively in the infected plant. Infestation with 1 and 2% ss and only 2% ss caused an increase in sucrose of root and shoot respectively in non infected plants. Additionally (wess) increased sucrose in both root and shoot. However, spraying the infected plant with (wess) or grow in soil supplemented with 2 and 5 or 5% only caused an increase in sucrose content in root and shoot respectively. Contents of polysaccharides increased in root of non infected plants and infected at 1 and 2% ss respectively while spraying with (wess) increased polysaccharides in the whole infected plants. Total carbohydrates content in non infected plant was significantly increased at 1 and 2% ss in root and shoot respectively, while in infected plants all treatments and only spraying with (wess) increased total carbohydrates of root and shoot respectively. On the other hand, total nitrogen of both root and shoot increased only at 5% ss as well as spraying with wess in infected plant.

Key words: Mushroom spent straw, metabolic changes botrytis fabae, broad bean

INTRODUCTION

Broad bean (*Vicia faba*) is one of the most important legume crops in many parts of the world. It is used as human food and for animal feeding as well as improving soil fertility. Chocolate spot caused by *Botrytis fabae,* is a widespread disease, occurring in almost all regions where broad bean is grown (Liang *et al.*, 1993; Koike, 1998).

Plant residues and in particular straw, contain large amounts of carbon source which can serve as substrates for production of microbial biomass and for biological nitrogen fixation by a range of free living diazotrophic bacteria (Rober and Ladha, 1995).

Spent mushroom substrates can be used as a soil fertilizer (Zhao *et al.*, 1994; Wuest *et al.*, 1995 ; Shukry *et al.*, 1999). In addition they can be used as carrier of *Rhizobium* and *Azotobacter* inoculants (Bahl and Jauhri, 1987).

Wilt disease of Cucumber caused by *Fusarium oxysporum f.sp.cucumerinum* could be delayed in presence of mushroom compost amendments in non sterile soil (Seo, 1986). Over the last decade, there have been reports of the use of water extract from composts for control of foliar diseases (Weltzien, 1991; Urban and Trankner, 1993; Elad and Shteinberg, 1994; Yohalem *et al.*, 1994 and 1995). Anaerobically fermented aqueous extracts of composts are among the biocontrol methods that have been suggested to replace synthetic fungicide. Using extracts from horse or cattle manure-based compost, Weltzien (1991) observed good control of gray mould (*Botrytis cinerea*) on strawberries, various powdery mildew and late blight of potato relative to untreated controls and in some cases, to fungicide preparations. At least partial control was reported for gray mould (*B. cinerea*) on tomato, pepper foliage and grape berries (Elad and Shteinberg, 1994) and grape leaves (Ketterer *et al.*, 1992) and for shoot blight on red pine caused by *Sphaeropsis sapinea* (Yohalem *et al.*, 1994). The addition into soil of composts reduced the effect of the pathogen on pea plants (*Pythium ultimum*) and avoided reductions of plant growth (Pascual *et al.*, 2002) One of the most important environmental and economic problems in the field of agriculture is the use of artificial fertilizers, synthetic growth regulators and pesticides. Synthetic substances may be considered as carcinogenic by long term application. Hence the new tendency nowadays is to use natural products as possible as to replace these synthetic substances. The aim of the work was to use the waste product of spent straw and its water extract after *Pleurotus floridanus* cultivation as natural biofertilizer in an attempt to control the Chocolate disease of broad bean by *Botrytis fabae*.

MATERIALS AND METHODS

Seeds of Vicia faba (Giza 402) susceptible to chocolate spot disease were used in this investigation. Seeds were supplied from Agronomy Research Institute Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt.

Two types of treatments were used in this investigation; the first was the infestation of air dried spent wheat straw (waste straw of mushroom cultivation) into the soil and the second was spraying the plants with water extract of spent straw.

The culture of *Pleurotus floridanus* was obtained from American type collection. The method used in cultivation was introduced by El-Fallal (1995) and can be summarized as : Spawn was prepared using sorghum grains at 54% moisture. Chopped wheat straw was soaked overnight and pasteurized by steaming for 7 h. After cooling, the straw was placed in plastic bags. Spawning was carried out at the rate of 5%. The latter were incubated for one month at room temperature. The plastic bags were perforated with nail size holes to provide aeration. The straw was sprayed with water twice a day. Mushroom harvest was carried out for 3 months. Then, the spent straw was air dried.

Spent straw were milled and sieved through 0.2 mm. sieves. Three different straw concentrations (1, 2 and 5% w/w) were added to clay soil as adopted by Shukry *et al.* (1999). Each concentration was mixed thoroughly with the soil and was placed in 20 cm diameter pot. The water extract of mushroom waste was prepared by the method suggested by Yohalem *et al.* (1996). Artificial infection of *Vicia faba* plants were carried out by spraying with the spore suspension of *Botrytis fabae* with or without the addition of the previous treatments. Control soil received no straw or extract. Four seeds of broad bean were planted in each pot. Five replicates for each treatment were carried out in the green house at the Faculty of Science, Mansoura University. The plant samples have been harvested at the seedling and flowering stages.

For every treatment the following growth criteria were measured : length of shoot and root, number of leaves, number of lateral roots, number of nodules, fresh weight of shoot and root. Metabolic aspects include carbohydrate contents (glucose, sucrose, polysaccharides) and total nitrogen in shoot and root were also detected.

Analytical procedures: The direct reducing value (DRV) was determined following the method of Nelson (Bell, 1955). The total reducing value was assayed after hydrolysis of sucrose with invertase. Polysaccharides were determined according to Younis *et al.* (1969). Total nitrogen and total soluble nitrogen were estimated by the conventional micro-Kjeldahl method (Pirie, 1955).

Estimation of cations in spent straw was determined according to the method described by Chapman and Pratt (1978). Flame-emission spectrophotometry was used for determining potassium and sodium while calcium was measured by atomic absorption spectrophotometry and total soluble salts (Piper, 1947).

Estimation of phenolic and indole compounds in spent straw has been determined according to the A.O.A.C. (1985) and Larsen *et al.* (1967) respectively.

RESULTS

The mushroom spent straw was analyzed chemically before application to the soil and the data was represented in Table 1.

Growth responses

Seedling stage: The supplementation of the soil by spent straw at concentration 1 and 2% caused significant increase and decrease in shoot and root length respectively (Fig. 1) in all plants. On the other hand, at 5% and water extract of spent straw opposite results were obtained.

It is noticed also that, infection by *B. fabae* caused decrease and increase in shoot and root length respectively. Addition of different concentrations of ss to the soil as well as spraying the plant with water extract of ss, increased both root and shoot lengths, also the number of leaves were significantly increased in all treatments in both infected and non infected plants.

At concentrations 2 and 5 %, there is significant increase in the number of nodules, lateral roots, fresh and dry weights of both root and shoot of all plants. On the other hand, spraying with water extract of spent straw increased fresh and dry weights of roots, while decreased fresh and dry weights of shoots.

Flowering stage: As shown in (Fig. 2) shoot and root lengths were highly significantly increased and significantly decreased respectively at all treatments in non infected plants. On the other hand, in case of infected plants a significant increase occurred at concentration 2 and 5% of spent straw (ss). Moreover, treatment with 2 % of ss showed an increase in the number of leaves in infected plants.

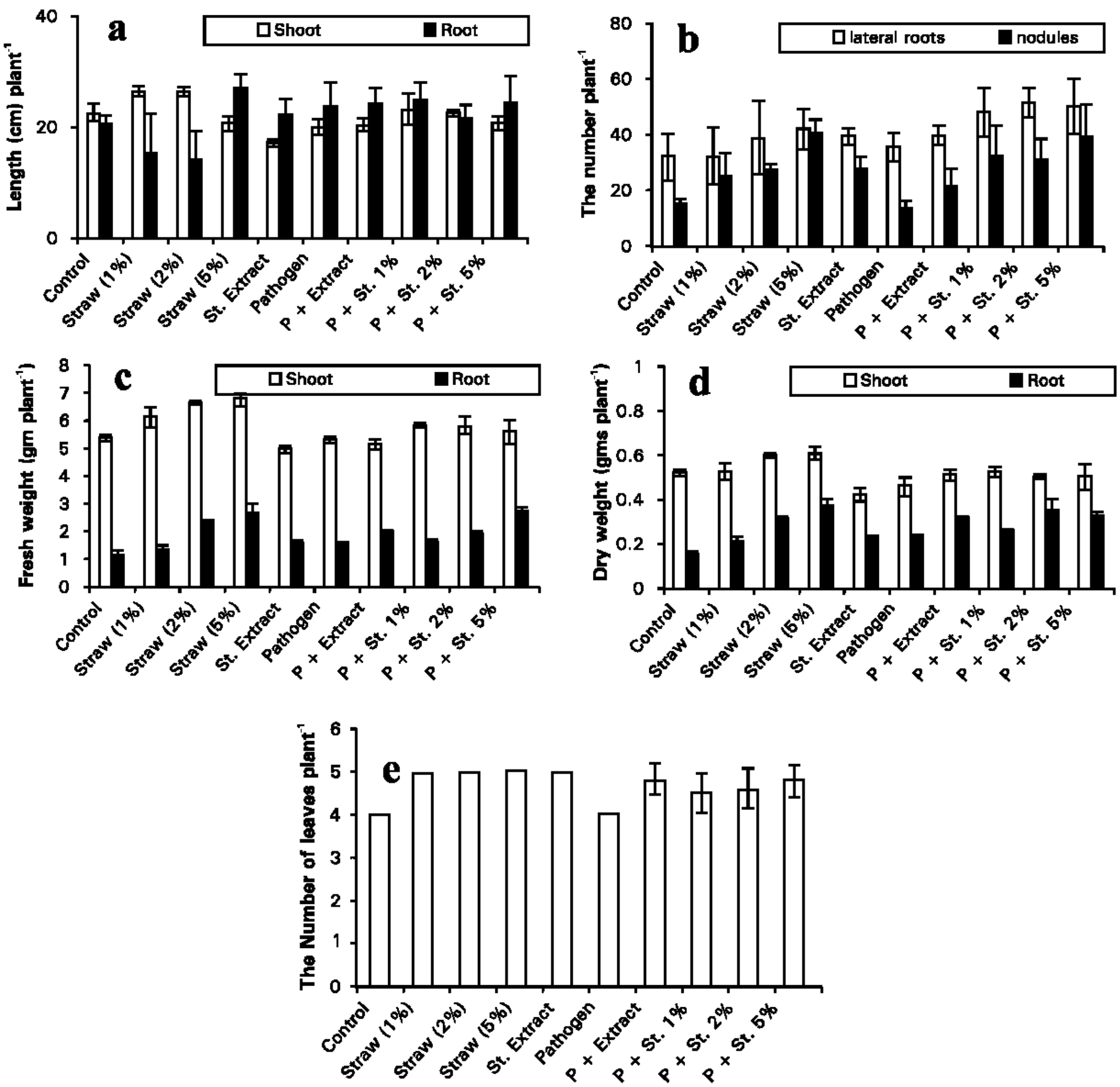

Fig. 1: Effect of spent straw (ss) and its water extract (wess) on growth parameters of broad bean infected by *Botryties fabae* at seedling stage. (a) Shoot and root length, (b) Number of lateral roots and nodules, Shoot and root fresh weight, (d) and dry weight, (e) number of leaves.

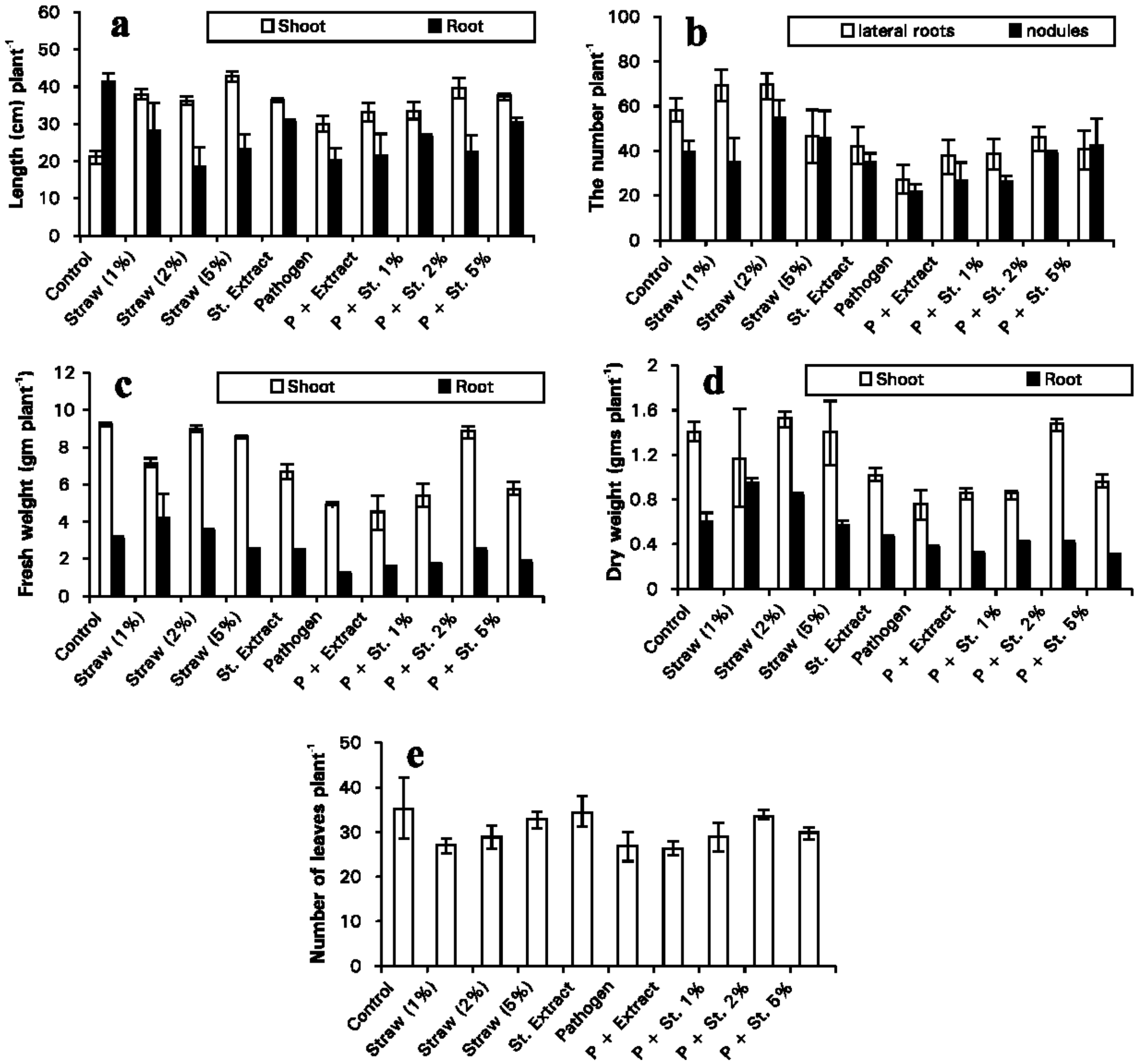

Fig. 2: Effect of spent straw (ss) and its water extract (wess) on growth of broad bean infected by *Botrytis fabae* at flowering stage (a) shoot and root length, (b) number of lateral roots and nodules, © shoot and root fresh weight, (d) and dry weight, (e) number of leaves

Table 1: Mushroom waste analysis

Parameters	Mushroom waste
zDRV	72.93±5.1
Sucrose (mg glucose/g D.wt.)	190.05±11.5
Polysaccharides	500.00±10.4
Total soluble nitrogen	2.20±0.3
Protein (mg NH4-N/g D.wt.)	10.58±1.1
C/N ratio	56:1
Potassium	10.80±2.1
Sodium (mM/g D.wt)	1.35±0.1
Calcium	0.14±0.01
IAA-oxidase (activity/g F.wt/hr.)	8.60±0.7
Phenolic compounds (μg/gD.wt)	294.30±24.1
Indole compounds (μg/gD.wt)	163.90±11.0

Generally, there is no significant difference between treatments, in their effects, on the number of both lateral roots and nodules, except at concentration, 2% of ss, a significant increase occurred in the number of nodules in non infected plants and in both parameters at concentrations 2 and 5% in case of infected one.

Increase in fresh and dry weight of root occurred at concentration 1 and 2% of ss in case of non infected plants and at concentrations 2 and 5% of ss in infected ones. Moreover, shoot fresh and dry weights were increased significantly at concentration 2% ss in all plants.

Change in carbohydrate content

Seedling stage: Regarding glucose content in non

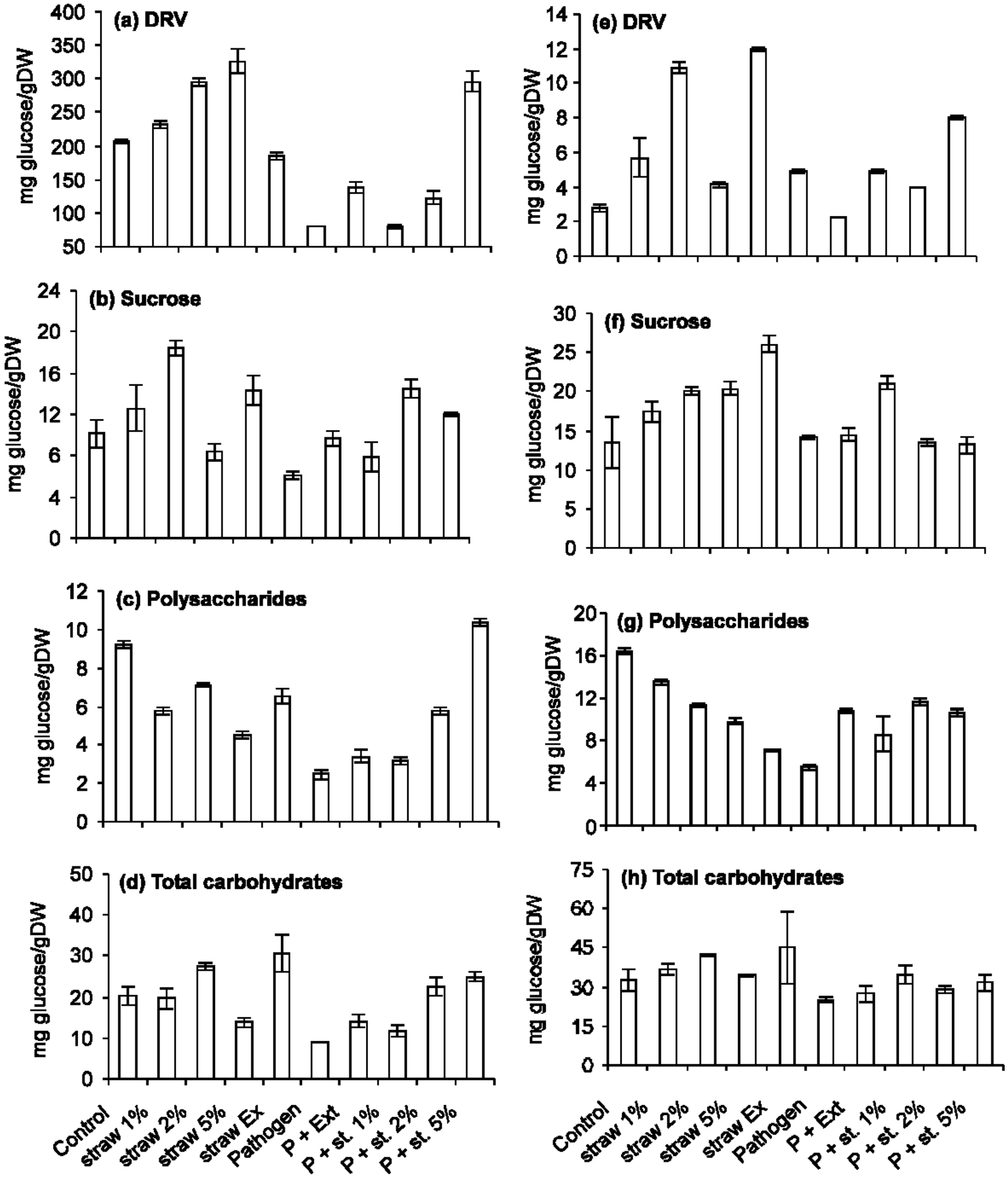

Fig. 3: Effect of spent straw (ss) and its water extract (wess) on carbohydrates content of broad bean (shoot and root) at seedling stage.

infected plants it was found that spraying leaves with water extract of ss and infestation of the soil with ss at concentration 2% showed a high significant increase in both root and shoot, while a non significant increase occurred at concentration 1 and 5%. On the other hand, highly significant increase occurred only at concentration 5% ss in infected plants (Fig. 3).

With respect to non infected plants, treatment with water extract of ss and also infestation of the soil with ss at concentration 2% highly significantly increased sucrose content in both shoot and root, while the other treatments showed non significant changes in sucrose contents except at concentration 5% which highly significantly increased the sucrose content only in shoots.

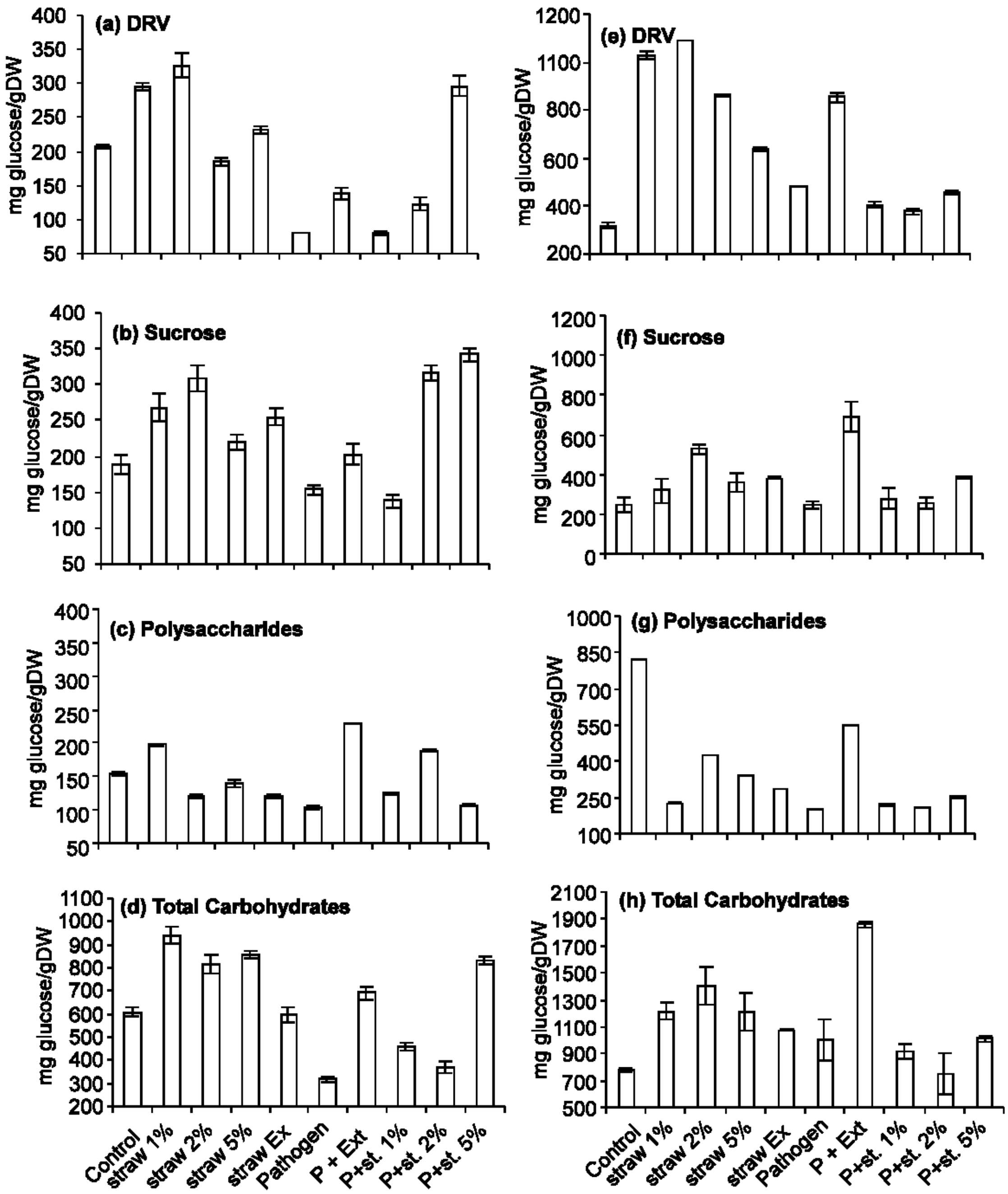

Fig. 4: Effect of spent straw (ss) and its water extract (wess) on carbohydrates content of broad bean (shoot and root) at flowering stage

High significant increase in sucrose contents of both shoot and root of infected plants occurred at concentration 1 and 2% ss respectively. On the other hand spraying with water extract of ss and also infestation the soil with concentration 5% ss induced significant increases in the roots only.

All treatments causing significant decrease in polysaccharides of shoot and root of all non infected plants. In contrast, high significant increase occurred at concentration 2 and 5% ss in both root and shoot, while spraying with water extract of ss showed significant increases in shoot and root of infected plants.

With regard to total carbohydrate, concentration 2% ss and water extract of ss caused highly significant increase in both shoot and root in non infected plants, while other treatments showed non significant changes in total carbohydrate. In case of infected plants all treatments showed increase in total carbohydrate especially at concentration 2 and 5% ss which induced a high significant increase in the case of root.

Flowering stage: With regard to non infected plants, high significant increase in glucose content of both root and shoot were induced by concentration 1 and 2% ss, but at 5% ss and upon spraying with water extract of ss the contents of glucose were significantly increased in shoot and no significant changes appeared to occur in case of root (Fig 4). In infected plants, treated with extract of ss and at concentration 5%, high significant increase in glucose occurred in shoot and root respectively. There is a significant increase at concentration 2% and upon spraying with extract of ss in root although the other treatments showed no significant effect in shoot.

Infestation with 1 and 2% ss caused highly significant increase in sucrose in case of root in non infected plant. On the other hand, highly significant increase in sucrose content occurred in shoot when the infected plants were sprayed with the extract (wess). Moreover, infestation with 2 and 5% caused high significant increases of sucrose in root and only at 5% ss in the case of shoot.

All treatments showed non significant effect on root polysaccharide content except at 1% ss in non infected plants and at 2% ss as well as upon spraying with the extract of ss in case of infected plants, when stimulation

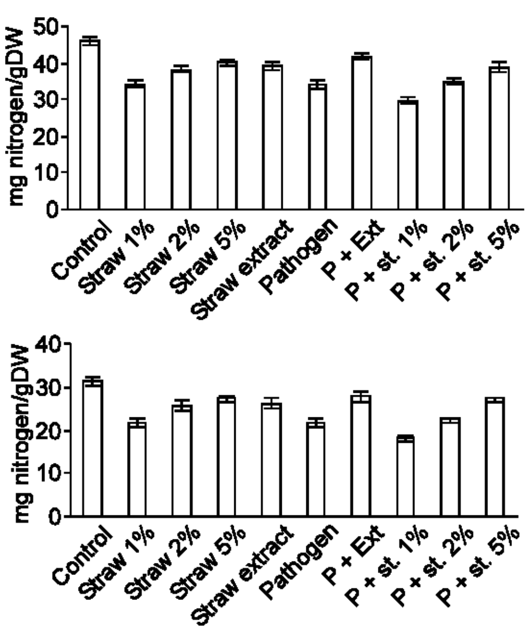

Fig. 5: Effect of spent straw (ss) and its water extract (wess) on total nitrogen contents of broad bean root and shoot at seedling stage

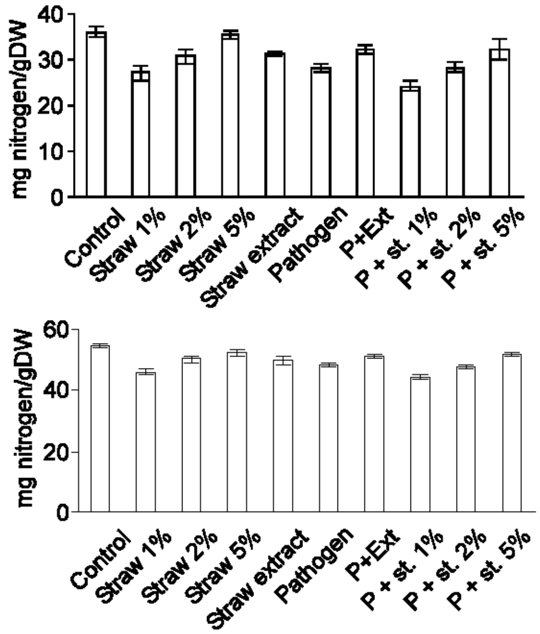

Fig. 6: Effect of spent straw (ss) and its water extract (wess) on total nitrogen contents of broad bean root and shoot at flowering stage

occurred. In case of non infected plants highly significant decrease occurred in shoot polysaccharides in all treatments while in case of infected plant high stimulation occurred only by spraying plant with extract of ss.

In non infected plants, the total carbohydrates of both shoot and root was highly significantly increased by infestation of soil with ss especially at 1 and 2%.

Also, high significant increase occurred in the total carbohydrates of the root of the infected plants at all treatments except at concentration 1% which induced a significant increase. On the other hand, in shoot, the high significant increase was induced only by spraying the plant with water extract of ss.

Change in total nitrogen: As shown in (Fig. 5 and 6) the total nitrogen in both shoot and root in the two stages (seedling and flowering) showed no significant change in total nitrogen in case of non infected plants as compared with the control ones. With respect to infected plants treatment with water extract of ss (wess) and also infestation of the soil with ss at concentration 5% significantly increased total nitrogen content in both shoot and root.

DISCUSSION

The analysis of *Pleurotus floridanus* spent straw revealed that it contains valuable amount of sugars, nitrogen, cations, hormones and some phenolic and indole compounds. These results are similar to those obtained by Shukry *et al.* (1999) and Shen and Shen (2001). The high values of these components resulted from the excretion of extracellular and intracellular enzymes by *P. floridanus* mycelial growth and fruit bodies (El-Fallal, 1990 ; Wood *et al.*, 1991).

Recent studies revealed that soil physical properties including bulk density, water- holding capacity, porosity and aggregate stability, have been changed favourably for plant production by compost application. These changes are mainly attributed to the addition of organic matter of composts (Abou El-Fadl *et al.,* 1995, Giusquiani *et al.,* 1995 ; He *et al.,* 1995).The light weight of organic matter dilutes the mineral fraction of soils, thus reducing soil bulk density (He *et al.,* 1992). Similarly, organic matter, particularly polysaccharides and other biopolymers, from composts can improve soil aggregate stability which in turn can change water holding capacity and porosity (He *et al.,* 1992). Increase in water holding capacity was attributed to the colloidal nature of composts (Guidi *et al.,* 1983) and to the high contents of fine particles (Wong *et al.,* 1983).

Results of the present investigation indicate that infestation the soil with spent straw at concentration 2 and 5% yielded better values for most of the growth parameters in both infected and non infected plants, whereas the highest values of fresh and dry weights of all plants were recorded. The induction of shoot length and weight of broad bean might have resulted from decrease in growth inhibitor and or increase the contents of auxins and gibberellins as concluded by Shukry *et al.* (1999), when they studied the effect of spent straw on cucumber plant. They also reported that the ratio 0.2% of spent straw exhibited the better growth of cucumber than 1 and 2%. Also, Alvarez *et al.* (1995) found that the dry matter of tomato plants grown on the composts was significantly higher than that of the control plants. In addition, the number of nodules and lateral roots were found to increase at concentration 2 and 5% ss. In this connection, Bahl and Jauhri (1987) concluded that wastes of mushroom cultivation can be used as a carrier for *Rhizobium* and *Azotobacter*.

The compost is widely used as a soil amendment to improve soil structure, provide plant nutrients, and facilitate the revegetation of disturbed or eroded soil (Cole *et al.*, 1995; Harmsen *et al.*, 1994 ; McNabb *et al.*, 1994). It was reported that refuse compost ratios 5-75% gave higher significant productivity of some vegetable species than those cultivated in sandy soil alone (Wong *et al.*, 1983). Also, ratio 25% v/v of compost showed highest yield of barley and oats (ECT, 1991). It was found that a moderate application of compost 2%, appears suitable for sensitive crops such as lettuce and spinach (Anid, 1986). In consequence the application of compost increased yields of corn (Duggan, 1973); potatoes (Purves and Mckenzie, 1974); tomatoes, barley and lettuce (Vlamis and Williams, 1972); oat and radish (Hortenstine and Rothwell, 1968) and sorghum (Hortenstine and Rothwell, 1973).

In this investigation there is also an increase in carbohydrates and nitrogen contents of all plants at concentration 2 and 5%, while their reduction in broad bean plants infected with *B. fabae* was detected. These results are confirmed by those obtained by Shukry *et al.* (1999) and Nofel (2000). On the other hand, spraying the shoot with water extract of spent straw (wess) and infestation the soil with 5% ss overcome this reduction. It has been known that protein is involved in the defence mechanism of plant diseases (Lucas, 1998), consequently spent straw increases the resistance of broad bean. Additionally plants grew better, which increases their tolerance to the presence of the pathogen. Meanwhile Yohalem *et al.* (1996) came to the same conclusion that a major inhibitory principle of the SMS extract is a low M.wt, heat stable, non protein metabolite produced by anaerobic microorganisms in the compost.

Alvarez *et al.* (1995) suggested that compost amendments to soils or used in container media may benefit plants through the effects of its humic fraction on the soil microflora and the plants. Shukry *et al.* (1999) reported that, addition of straw in the soil caused an increase in the number of total bacteria, actinomycetes and fungi of the rhizosphere. Vallini *et al.* (1993) have reported an increase in the number of total aerobic bacteria and actinomycetes in the laurel caused by humic acid treatment. In addition, humic substances may affect the plant biochemical process (Vaughan *et al.,* 1985) and /or bacteria (Visser, 1985a,b). Changes in metabolism process of plants may induce a resistance to certain phytopathogens. Foliar diseases also may be affected by composts incorporated into soil (Weltzien, 1991). Ranyanathan and Selvaseelan (1994) observed that green gram seed yield was increased in plots previously supplied with mushroom spent rice straw.

It can be concluded from this study that infestation the soil with 2 and 5% spent straw and spraying the broad bean plant with water extract of spent straw have two roles: firstly, stimulating the plant growth so it can be used as biofertilizer, secondly, reducing the suppressive effect of *B. fabae* on the plant. The prospect for the future is good for the application of wastes from mushroom cultivation because of the lessened availability of chemicals to fertilize and protect crop plants.

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