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ORIGINAL ARTICLES

Safety chemicals as tools for improving the nutritional status and inducing phyto-resistance of common beans grown in soil infected with *Sclerotium rolfsii*

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ABSTRACT

Sclerotium rolfsii is one of the important pathogens which attack common bean plants causing serious damages and unexpected losses in yield. Effect of three amendments of no environmental risk i.e. paclobutrazol (PBZ), sulphated canola oil (sulphex) and humic acid on improving nutritional status of plant and reducing the invasion of common beans by *Sclorotium rolfsii* and hence increasing the pod yield were studied through a field experiment conducted for two successive years. Investigating the bio-defense mechanisms against infection with *S. rolfsii* in comparison with the effect of the fungicide Vitavax-thiram on the plants infected with *Sclorotium rolfsii* was also a matter of concern. The percentage of plant survival and pod yield increased significantly due to application of the amendments whose effects were significantly higher in the second growing season than in the first one. Significant increases in the uptake of N and P occurred due to these amendments. The uptake of Cu and Zn in the first growing season exceeded significantly the corresponding ones in the second growing season. PBZ stimulated the activity of chitinase enzymes as a defense mechanism against invasion of *S. rolfsii*, humic acid stimulated the activity of peroxidase enzymes and sulphex increased the activities of chitinase, peroxidase and polyphenol oxidase enzymes. Therefore, humic acid and PBZ are recommended for inducing phytoresistance of common bean grown in soil infected with *S. rolfsii* and improving its nutritional status without causing any environmental hazards.

Key words: *Sclerotium rolfsi*i; humic acid; sulphated canola oil; paclobutrazole; nutrient uptake.

Introduction

Common bean (*Phaseolus vulgaris* L.) plant is an important source of protein, minerals and vitamins (Gepts *et al.*, 2008) which can grow in low fertility soils using minimal technical inputs (Weckx and Clijsters, 1996). Common bean is highly consumed worldwide especially in the developing countries (Reyes Moreno *et al.*, 1993). *Sclerotium rolfsii*, the causal of white rot, is one of the important pathogens which attack common bean plants mainly under high temperature and high moisture conditions (Jin-Hyeuk Kwon *et al.*, 2004), causing serious damages to the plants and high losses in their yield (Embaby, 2006). The mechanism of pathogenesis takes place through secretion of large amounts of oxalate (Lehner *et al.*, 2008) and other enzymes which degrade plant cell wall (Punja *et al.*, 1985). Afterwards, *S. rolfsii* attacks plant tissue directly (Prasad and Naik, 2008). Fungicides are commonly used for controlling such a pathogen and this is costly and has a negative environmental implication (Wahab, 2009). Therefore, trying other safely environmental chemicals instead of the common fungicide chemicals for controlling plant pathogen *S. rolfsii* would be favored (Mukerji and Ciancio, 2007). Sulphated canola is suggested to be one of the substitutes where the elemental sulphur was used as an efficient fungicide (Williams and Cooper, 2004) and the volatile compounds produced through the decomposition of oil in soil could be toxic for *S. rolfsii* (Kottearachchi *et al.*, 2012). Thus a sulphur containing oil can sucessfully be used in controling the pathogen (Pohoreski, 2004). On the other hand, vegetable oils e.g. canola, when applied at low concentrations, could stimulate the activity of soil bacteria including N-fixing bacteria, being a primary sources of energy (Bonnett *et al.*, 2012). Furthermore, sulphur can reduce soil pH and increase the solubility and availability of soil micronutrients and phosphate for plants (Chien *et al.*, 2011). Pohoreski (2004) recommended application of diluted sulphur containing oil for crops is about 100 L ha^{-1} (5g L^{-1}).

Paclobutrazol (PBZ) compounds are potential growth regulators which decrease shoot elongation and biomass production and consequently lessen plant nutrient requirements (Asín *et al.*, 2007) whithout affecting yield quantity or quality (Abdel Rahim *et al.*, 2011). Moreover, PBZ has fungicide properties for some soil-born pathogens (Bolu and Cimen, 2006; Gopi *et al.*, 2007). This hormon has no hazardous effect on soil microbial population or activity (Silva *et al.*, 2003) and can be bio-degradated by microbes (Chen *et al.*, 2010). These agrochemicals are therefore suggested to be a suitable substitute for the fungicides used for controlling the

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pathogen. It also can be used either through foliar spray or through the soil (Bolu and Cimen, 2006).

Providing the grown plants with adequate amounts of nutrients could increase their resistance to pathogens (Dordas, 2009). Humic acid can increase nutrient uptake by the grown plants and stimulate plant enzymatic activity (Chen and Solovitch, 1987) as well as improve plant growth (Nardi *et al*., 2002). Treating plants with humic acid up to 300mg L^{-1} can effectively induce the plant resistance against root rot invasion (Loffredo and Senesi, 2009), probably through activating microorganisms antagonistic to plant pathogens (Shigemitsu, 1984). Yigit and Dikilitas (2008) reported that the application of more than one dose of humic acid at a rate of 80 mg L^{-1} stimulated tomato infection with fusarium in highly infected soils. There is lack in information about the effect of the time of application of humic acid on improving the nutritional status of the plant without stimulating the white rot pathogen. Smith *et al*. (1986) found that *S. rolfsii* invasion took place after the death and collapse of the seedling infected cells. Therefore, humic acid was applied, in this study, during sowing the seeds and before seedling to ensure low activity of *S. rolfsii* in soil

The current research aimed at trying paclobutrazole plant growth regulator, sulphated canola oil and humic acid as safer and cheaper substitutes for the fungicide chemicals through improving the nutritional status of common bean and hence inducing its phyto-resistance against infection with *S. rolfsii*. This study is an integrated study including pot and field experiments carried out for two successive seasons.

Materials and Methods

Soil of study:

A surface soil sample (0-30 cm) was collected from the experimental farm of the Faculty of Agriculture at Moshtohor, Benha university prior to the field study in 2009 and 2010 seasons. This sample was air dried, crushed, sieved to pass through 2 mm and analyzed for physical and chemical properties according to the standard methods outlined by Page *et al.* (1982) and Klute (1986). Physical and chemical properties of the studied soil are shown in Table 1.

Table 1: Physical and chemical properties of the studied soil.

Soil characteristics	season		Soil characteristics	season	
	2009	2010		2009	2010
Coarse sand (%)	2.00	4.2	Organic matter (g kg^{-1})	1.51	1.35
Fine sand (%)	23.41	19.1	pH	7.83	7.67
Silt (%)	33.45	30.3	EC (dS m^{-1})	2.43	2.17
Clay (%)	41.14	46.4	Total N (g kg^{-1})	1.15	5.14
Textural class	Clay loam	Clay loam	Available P (mg kg^{-1})	43.0	12.0
$CaCO_3$ (g kg^{-1})	25.0	22.0	Available K (mg kg^{-1})	976.0	688.0

pH: 1:2.5 soil :water suspension; EC: saturation paste extract

Isolation and identification of the stem rot pathogen:

A white moldy layer with small, smooth and brown sclerotia was detected in the parts of common beans in contact with the soil. It was initially identified as *Sclerotium rolfsii* infection according to Schwartz *et al*. (2005) and FAO (2007). A further confirmation was obtained through examining the morphological characteristics of this pathogen identified under microscope by the Department of Fungal Taxonomy, Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

Seeds and the used amendments preparation:

The seeds of common bean plants (*Phaseolus vulgaris* L. cv. Bronco) were obtained from the Vegetable Crops Research Deptartment, Agricultural Research Center (ARC), Giza, Egypt. Paclobutrazol (PBZ) plant growth retardant (250 g L^{-1}) was obtained for use in this study from Syngenta Co. under the commercial name “Cultur”. A solution of PBZ of 40 mg L^{-1} was prepared using deionized water. Canola oil (100% pure oil) was obtained from Al-Ghurair Foods (LLC), Dubai under the trade name “Jenan”.

Preparation of sulphated canola oil referred to as “sulphex” was done according to Pohoreski (2004) by adding 4.77 mL of concentrated H_2SO_4 to three kilograms of canola oil and the mixture was allowed to stand for 18h. Afterwards, 3.88 L of NaOH (3*N*) was added slowly to the mixture and left for 24h. The sulphated oil was then removed from the top of the mixture, diluted with deionized water at a volume ratio of 1:4 and the pH of the resultant solution was adjusted to 4 using 3*N* NaOH. Fifty milliliters of the prepared "sulphex” solution was then diluted with deionized water to 10 L to obtain sulphex (5g kg^{-1}).

Humic acid (analytical grade) with a purity of 99% was obtained from Fluka Chemika). A solution of 400 mg L^{-1} was prepared from humic acid using deionized water.

J. Appl. Sci. Res., 9(8): 5283-5293, 2013

The preliminary greenhouse experiment:

A pot experiment was conducted in the greenhouse of the Department of Plant Pathology, Faculty of Agriculture, Benha University, Egypt to investigate the effects of the plant growth regulator PBZ, humic acid and sulphex on controlling the white rot decease of the bean plants grown on a soil infected with *Sclerotium rolfsii* and at the same time improving the plant performance and green pod yield. To attain these aims, plastic pots (25 cm diameter) were uniformly packed with sterilized air-dried soil infected artificially with *Sclerotium rolfsii* at a rate of 3.0% (w/w). Five seeds of common bean (*Phaseolus vulgaris* L., cv. Bronco) were sown in each pot and treated with either of (1) paclobutrazol (PBZ) (40 mg L^{-1}) applied to soil at a rate of 20 mL per pot (PBZ, T_1), (2) humic acid applied to soil at a rate of 200 mL humic acid solution (400 g L^{-1}) per pot (HA, T_2), (3) sulphex oil applied to soil at a rate of 20 mL of the (0.5%) diluted sulphex solution per pot (Cnl, T_3), (4) the Vitavax-thiram fungicide applied at a rate of 15 g kg^{-1}(T_4) and (5) the control treatment (T_5) . Soils surfaces were covered with acid washed sand to keep the soil warm. All pots received NP fertilizers as recommended by the Minstry of Agriculture i.e. ammonium sulfate fertilizer (20.5% N) at a rate of 65 mg N kg^{-1} , calcium super phosphate (6.55%P) at a rate of 12 mg P kg^{-1} and potassium sulfate (20%K) at the rate of 40 mg K kg^{-1}. The experimental design was a randomized complete block with three replicates and kept under greenhouse conditions. At the 15^{th} , 30^{th} and 60^{th} days after planting (DAP), the percentages of pre- and post-emergence damping-off as well as healthy survived plants were recorded. The bean plants were sampled 35 days after planting for determining the activities of plant enzymes. After 60 days, the whole plants removed gently from soil and their fresh weights were recorded. The plant materials were then oven dried at 70 C for 48 h and their dry weights were recorded.

The field experiment:

A field experiment was conducted in the experimental farm of the Faculty of Agriculture, Benha University, Egypt during the period between February and March of the two successive seasons 2009 and 2010. The experimental design was a randomized complete block in three replicates. The area of the plot was 10.5 m^2. Seeds of common beans were sown in all the experimental plots. Paclobutrazol (40 mg L^{-1}) was added at a rate of three m^3 ha^{-1}. Sulphex oil (0. 5%) was added at a rate of one m^3 ha^{-1}. Humic acid was added at a rate of 16 kg ha^{-1}. Vitavax-thiram was used at a rate of 3g kg^{-1} seeds. All treatments were added within the top 20 cm of soil surface during seed sowing. N was added to the experimental field at a rate of 48 kg N ha^{-1} (as ammonium sulfate, 205 g N kg^{-1}) + 31 kg P ha^{-1} (as calcium super phosphate, 65.5g P kg^{-1}) +100 kg K ha^{-1} (as potassium sulfate, 400 g K kg^{-1}). The usual agricultural practices were followed as recommended. At the 15^{th} , 30^{th} and 90^{th} days after planting (DAP), the percentages of pre- and post-emergence damping-off and the survived healthy plants were recorded, respectively. At physiological maturity, whole plants were removed from the middle row of the experimental plot gently to avoid root damage and washed under gentle current of tape water and then rinsed twice with deionized water. Plants were then separated into roots, shoots and green pods and oven dried at 70 C for 48 h.

Soil and plant analyses:

The dried plant materials were grounded sieved to pass though a 2-mm micromill then digested with a mixture of concentrated sulphuric (H_2SO_4) and perchloric ($HClO_4$) acid (1:1) as outlined by Peterburgski (1968). Total nitrogen in the digest was determined using micro –Kjeldahl technique whereas total phosphorus was determined photometerically according to AOAC (1980) while Cu and Zn concentrations were determined in the plant digest by Atomic Absorption Spectrophotometer 210 VGP. Peroxidase and polyphenol oxidase enzymes were determined in the plants collected from the green house experiment according to the method described by Allam and Hollis (1972) and Matta and Dimond (1963), respectively, while chitinase activity was determined colourimetrically according to Boller and Mauch (1988)

Statistical analysis:

Data collected were analyzed with the statistical analysis system (CoStat Pro. 2005). All multiple comparisons were first subjected to analysis of variance (ANOVA). The differences between the mean values of various treatments were compared by Duncan's multiple range test (Duncan, 1955).

Results and Discussion

Effects of humic acid, sulphex and PBZ on common bean plants under the greenhouse conditions:

Effects on root and shoot fresh weights (g plant^{-1}) , and the percentage of plant survival:

Results shown in Fig. 1 and Table 2 reveal that treated plants showed increased fresh weight of roots and shoots. The highest increases in shoot fresh weight were recorded for plants treated with the fungicide and sulphex. On the other hand, no significant differences were detected in root fresh weight between the different treatments and the fungicide treatment. Significant increases occurred in the percentage of plant survival due to application of the used treatments compared with the control. No significant difference was detected between the fungicide treated plants and either of the humic or canola treated ones, while the PBZ treatment recorded the least significant increases in the percentage of plant survival compared to the control.

Table 2: *Phaseolus vulgaris* survival percentage and growth in soil infected with *Sclerotiom rolfsii* as affected by paclobutrazol , humic acid, sulphated canola oil and Vitavax-thiram fungicide under greenhouse conditions.

	Root wt (g plant^{-1})		Shoot wt (g plant^{-1})		Damping off		Survival plant %
	Fresh weight	Dry weight	Fresh weight	Dry weight	Pre%	Post%	
Paclobutrazol	8.15 ^{a}	3.13 ^{a}	44.78 ^{b}	11.33 ^{b}	22.73 ^{b}	8.80 ^{b}	68.47 ^{b}
Humic acid	7.77 ^{a}	3.61 ^{a}	43.64 ^{b}	11.25 ^{b}	11.60 ^{c}	8.40 ^{b}	80.00 ^{a}
Sulpex	8.53 ^{a}	3.41 ^{a}	45.58 ^{ab}	11.50 ^{b}	11.13 ^{c}	9.07 ^{b}	79.80 ^{a}
Fungicide	8.69 ^{a}	3.18 ^{a}	52.59 ^{b}	13.86 ^{a}	11.53 ^{c}	10.33 ^{b}	78.13 ^{ab}
Control	4.93 ^{b}	2.46 ^{b}	23.47 ^{c}	7.73 ^{c}	57.70 ^{a}	21.24 ^{a}	21.69 ^{c}

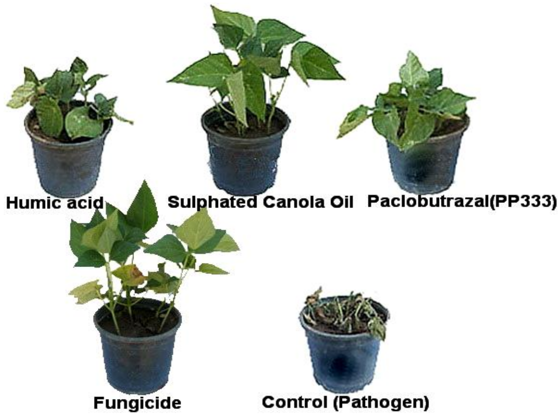

Fig. 1: *Phaseolus vulgaris* growth in soil infected with *Sclerotiom rolfsii* as affected by paclobutrazol, humic acid, sulphated canola oil and Vitavax-thiram fungicide under greenhouse conditions.

Effects on chitenase, peroxidase and polyphenol oxidase enzymes ion bean plants:

Table 3 reveals that treated plants showed increases in the activities of enzymes chitenase, peroxidase and polyphenol oxidase. The activities of the fungicide treated plant enzymes were taken as references in this study for the enzymatic activity of the plant which grows in soil infected mildly with *S. rolfsii* because of the well known effect of the used fungicide on reducing the phathogen invasion for common beans and hence improving the percentage of plant survival, without extra enzymatic bio-defense. The sulphex treatment recorded the highest increases in chitenase, peroxidase and polyphenyl oxidase activities. The humic acid treatment recorded pronounced increases in chitinase and peroxidase enzymes; whereas the PBZ treatment showed pronounced increases over the fungicide treatment only in chitinase activity.

Table 3: Chitenase, peroxidase and polyphenol oxidase activities in bean plants grown in soil infected with *Sclerotiom rolfsii* as affected by humic acid, sulphated canola oil , paclobutrazol and Vitavax-thiram fungicide under the greenhouse conditions.

Treatments	Chitenase	Peroxidase	Polyphenol oxidase
Paclobutrazol	16.50^{c}	10.85^{b}	17.10^{c}
Humic acid	10.50^{b}	16.31^{a}	18.18^{c}
Sulphex	25.80^{a}	16.66^{a}	32.40^{a}
Fungicide	7.80^{d}	10.50^{b}	27.00^{b}
Control	3.00^{e}	3.50^{c}	13.50^{d}

1- Chitinase activity was expresses as mM N-acetyl glucose amine equivalent released / gram fresh weight tissue / 60 minutes.
2- peroxidase activity was expressed as the change in absorbance (O.D) / minute/gram fresh weight.
3- The polyphenoloxidase activity was assayed as the change in absorbency (O.D) / minute/gram fresh weight.

J. Appl. Sci. Res., 9(8): 5283-5293, 2013

Effects of humic acid, sulphex and PBZ on common bean plants under the field conditions:

Effects on the percentage of survival of common bean plants:

Table 4 shows that treated plants exhibited increases in the percentage of plant survival during the first growing; especially the sulpex (T2) and fungicide (T4) treatments. However, during the second growing season, no significant differences were detected in plant survival among the treated ones and their magnitudes remained much higher than the non-treated plants.

Effect on values of nutrient uptake by plants and their distribution in the different plant parts:

Effect on N –uptake:

Nitrogen uptake by common bean plants increased significantly owing to the application of PBZ, suphated canola oil and humic acid treatments during the first growing season (Fig 2a and 2b). The highest N uptake was recorded for the plants treated with humic acid (with no significant difference with the fungicide treated plants).; whereas the lowest N uptake was recorded for the plants treated with sulphated canola oil. However, during the second growing season, higher significant increases in N uptake occurred due to PBZ, suphex and humic acid.

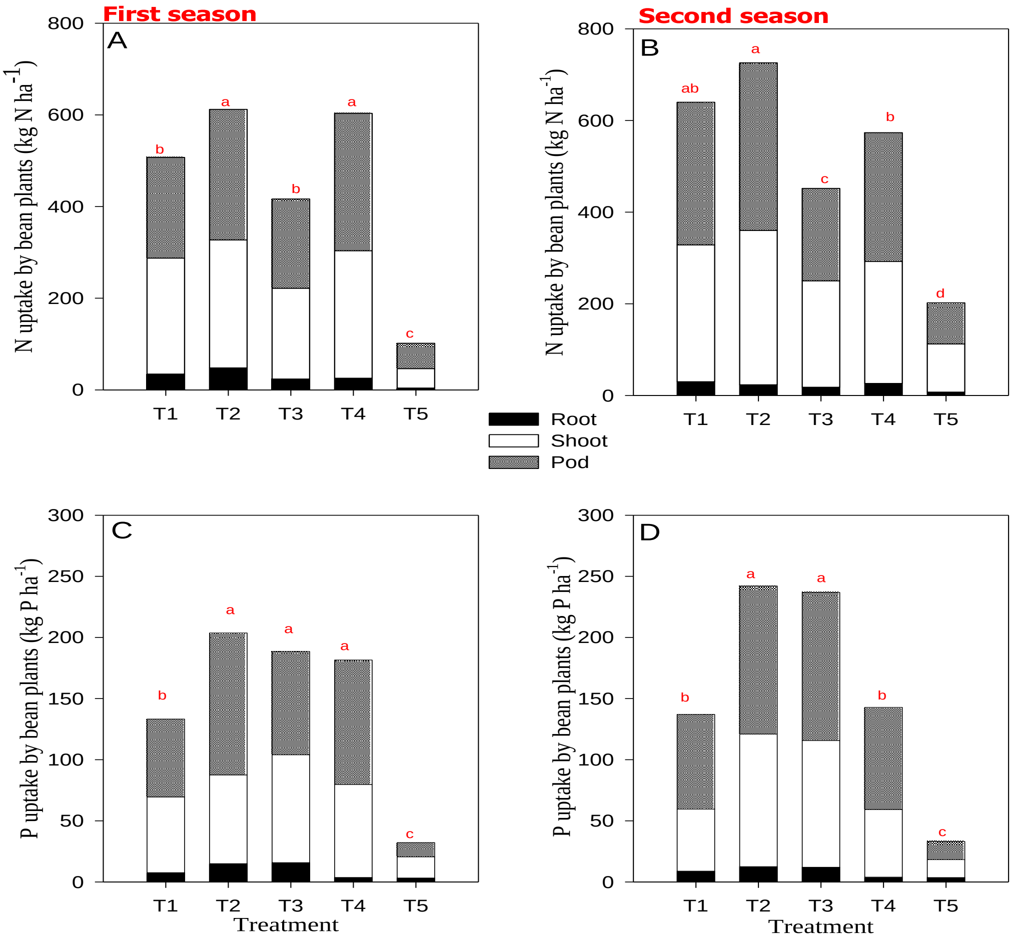

Fig. 2: N and P uptake by bean plants and their distribution in the different plant parts : PBZ (T_1),:humic acid (T_2), sulphex (T_3), fungicide (T_4), control (T_5).

Table 4: Disease incidence and disease severity in bean plants as affected paclobutrazol, humic acid, sulphated canola oil , and Vitavax-thiram fungicide under the field conditions.

Treatments	First season			Second season		
	Damping off		Survival plant %	Damping off		Survival plant %
	Pre%	Post%		Pre%	Post%	
Paclobutrazol	18.48 b	7.63 c	73.89 b	22.92 b	5.56 b	71.53 a
Humic acid	13.77 b	13.15 b	71.83 b	22.92 b	4.17 b	72.92 a
Sulphex	12.50 b	9.79 c	77.70 ab	24.97 b	3.13 b	71.88 a
Fungicide	11.32 b	8.04 c	80.50 a	22.92 b	4.17 b	72.92 a
Control	40.63 a	21.46 a	37.92 c	46.53 a	12.73 a	40.74 b

Effects on P-uptake:

Significant increases in P uptake by common bean plants occurred due to application of the different materials during the first growing season (Fig 2c and 2d). In this concern, no significant differences in P uptake were detected between plants treated with fungicide and those treated with either of humic acid or sulphonated canola oil. The least increases in P uptake were recorded for the plants treated with PBZ. During the second growing season, significant increases in P uptake occurred for both the plants treated with humic acid and sulphonated canola; whereas, significant decreases were noticed for PBZ and fungicide treated plants.

Effects on Cu-uptake:

Figs 3A and 3B illustrate the increases in values of Cu uptake by bean plants due to the different treatments during the first growing season. Such increases seemed to be more significant for plants treated with humic acid and sulphex as well. The lowest increases occurred in plants treated with PBZ. During the second growing season, significant reductions in Cu uptake occurred due to all the treatments with lower Cu translocation to the green pods. Moreover, Copper uptake due to PBZ, humic acid, sulphex were higher than those obtained with the fungicide treatment during the second growing season.

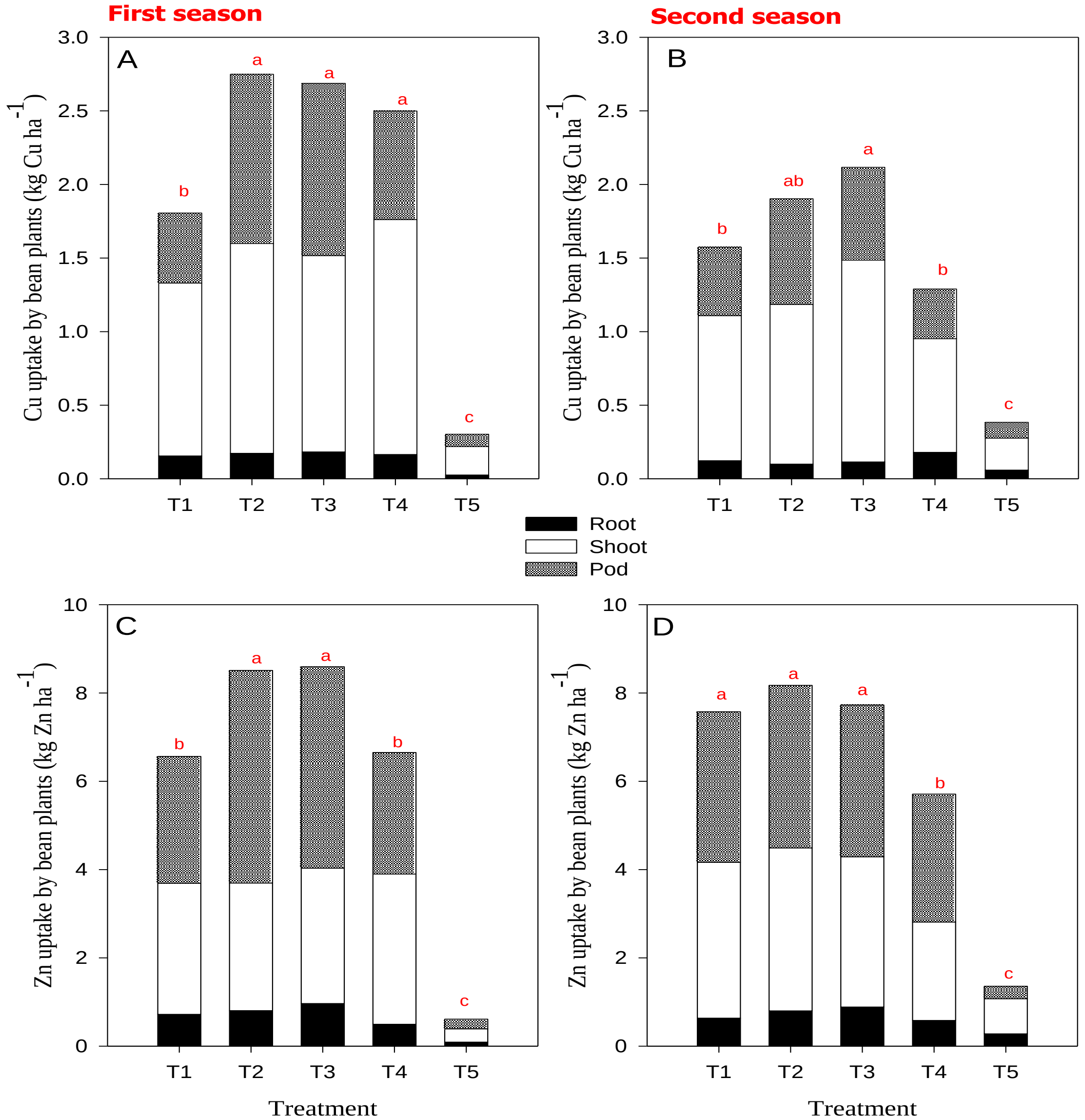

Fig. 3: Cu and Zn uptake by bean plants and their distribution into the different plant parts : PBZ (T_1),:humic acid (T_2), sulphex (T_3), fungicide (T_4), control (T_5).

Effects on Zn-uptake:

Figs 3C and 3D demonstrate the increases in Zn uptake by bean plants occurred due to PBZ, humic acid, sulphex treatments during the first growing season. The increases recorded due to humic acid and sulphex treatments exceeded those attained due to the fungicide one, while no statistical differences were found between PBZ and fungicide treatment. No difference occurred in Zn uptake during the second growing season due to humic acid and sulphex treatments whereas, significant increase in Zn uptake occurred due to PBZ treatment and on the other hand significant decrease was occured due to the fungicide treatment.

Effects on the different growth parameters:

Table 5 shows significant increases in plant biomass and pod yield due to application of each of PBZ, sulphex and humic acid. The increases in these parameters were higher during the second growing season than the first growing one. The highest pod yield in the first growing season was recorded due to humic acid and the fungicide treatments. During the second growing season, the pod yields attained due to humic and PBZ treatments exceeded the corresponding one achieved due to the fungicide treatment. Although the pod yield achieved due to sulphex was higher than that achieved due to the fungicide treatment in the second season, such a difference was statistically insignificant.

Table 5: Common bean growth parameters as affected paclobutrazol, humic acid, sulphated canola oil , and Vitavax-thiram fungicide under the field conditions.

Treatments	First season			Second season		
	root	Shoot	pod	root	Shoot	pod
PP333	1485.57^{a}	7959.84^{ab}	6075.03^{b}	1857.00^{b}	9863.79^{a}	8928.90^{a}
Humic acid	1486.56^{a}	6384.60^{b}	7246.59^{a}	1601.07^{c}	9628.44^{a}	9288.42^{a}
Sulpex	1746.51^{a}	8134.80^{ab}	6008.91^{b}	1761.60^{b}	9604.47^{a}	8310.15^{ab}
Fungicide	1561.05^{a}	11319.30^{a}	7804.17^{a}	2189.91^{a}	10621.23^{a}	8042.97^{b}
Control	340.14^{b}	1591.95^{a}	1662.66^{c}	758.73^{d}	3508.20^{c}	3286.38^{c}

Discussion:

This study aimed at investigating the effects of treating common bean plants grown in soil infected with *S. rolfsii* with each of the plant growth regulator (PBZ), sulphex and humic acid on reducing the negative impacts of *Sclorotium rolfsii* pathogenesis, improving the nutrient status of the grown plants and increasing the productivity of common bean pod yield. The PBZ is a growth retardant, under normal condition, can reduce plant shoot biomass (Sharma *et al.*, 2011; Tran and Constabel, 2011) and therefore directs more nutrients towards seed and fruit production (Yim *et al*., 1997). Our records indicate, under soils infected with *S. rolfsii*, that treating common bean plants with PBZ led to significant increases in the percentage of plant survival and the plant growth parameters i.e. root, shoot and green pod fresh weights. These parameters recorded significantly higher increases in the second growing season than those attained in the first growing one. Such results point out to the effectiveness of PBZ on increasing plant resistance towards infection with *S. rolfsii*. Martínez *et al.* (2010) reported that PBZ can be used effectively as a fungicide; however its mode of action is not clear. Uptake of N and P increased significantly in the plants treated with PBZ during the first growing season followed by further significant increases in NP uptake during the second one. Studies by Meng *et al.* (2012) indicate that NP content in leaves of 18-year-old Muye red bayberry trees increased with the application of PBZ. This indicates that improving the nutritional status of the grown plants could effectively reduce the decease incidence on the grown plants and, at the same time, increase their resistance towards the pathogen. Significant increases in Cu and Zn uptake occurred in the first growing season which maintain high concentrations of these nutrients in plant tissues; however, during the second growing season, significant reductions in Cu uptake occurred and its concentrations in plant tissue became lower. In case of Zn, its uptake increased slightly during the second growing season; however such increases were associated with lower concentrations of Zn in plant tissue according to the dilution effect phenominon. The Cu and Zn nutrients might act as bio-defenders againest the pathogen infection as they increased in plant tissue during the first growing season that was caracterized by higher severe pathogenity. The decrease in plant tissue during the second growing season occurred with decreasing the severity of the pathogen. Stimulaing the activity of chitinase enzyme was also one of the consequences of plants treated with PBZ towards the pathogen. Chitinase is considered one of the mechanisms responsible for controlling fungal plant pathogens (Patil *et al.*, 2000) while it hydrolyzes the chitin of the cell walls of the fungal pathogen (Natsir *et al.*, 2002), beside of being a chitin-binding domain (CBD) (Iseli *et al.*, 1993). This enzyme might have specific requirements of both Cu and Zn ions for its activity (Ghanem *et al.*, 2010).

Application of humic acid and sulphex to soil led to significant increases in the percentage of plant survival and promoted the plant growth (root and shoot) and pod yield. Further increases in the percentage of plant survival and the pod yield were obtained during the second growing season. Thus, it can be concluded that both the humic acid and sulphex cooperated effectively in increasing plant resistance towards *S. rolfsii* invasion. Significant increases in NP uptake occurred due to amending soil with each of humic acid and sulphated canola oil. Humic acid might have chelated metals that fix phosphate in soil and therefore released P free in soil solution (Cornish, 2009) and may have inhibited further formations of insoluble calcium phosphates (Paul Grossl *et al.,* 2009). In case of sulphated canola oil, the acidity of this amendment might account effectively for increasing the solubility of P in soil. Pradhan and Sukla (2005) found that approximately 95–99% of P is found in soil in the form of insoluble phosphates and the decrease in soil pH would effectively increase P solubility and availability in soil. Such increases in available P would stimulate nitrogen-fixing bacteria in soil (Rondon *et al.,* 2007) which would lead to concurrent increases in the uptake of N and P by common bean plants. Significant increases in Cu and Zn uptake by bean plants also occurred owing to the application of each of humic acid or sulphex. These increases exceeded those obtained due to PBZ treatment. Lower uptake values of Cu and Zn occurred during the second growing season. Such results mean that Cu and Zn might account effectively for reducing the plant resistance towards the pathogen invasion. Probably, Zn and Cu availability increased in soil amended with humic acid during the growing season in the form of soluble Zn -, and Cu-humate complexes. In this concern, the applied humic acid might coordinate with soil Cu forming soluble Cu (II)-humic complexes (Pandey *et al.*, 2000) which remain stable in soil solution for long periods of time (Hampp and Tarkka, 2009). Also, Zn humate is considered an efficient Zn supplier for eliminated Zn-deficiency symptoms(Ozkutlu *et al*., 2006). On the other hand, the application of sulphex might reduce soil pH and thus increased Zn and Cu solubility in soil. Further reports indicated that acidifying soil can improve Zn availability (Muhammad *et al*., 2012) and Cu availability (Nascimento *et al*., 2003).

High concentrations of available Cu in soil can suppress the growth of the white rot fungi, *S. rolfsii*, because of their low requirements and tolerance for Cu compared with higher plants (Graham and Webb, 1991). It was also reported that copper has high affinity for the nitrogen organic compounds i.e. proteins, causing their denaturation (Rusjan, 2012), therefore high concentrations of available Cu in soil can be chelated with fungal proteins and thus result in reduction in their ability to reproduce and lessen their ability for plant infection. Moreover, the pathogen exudates i.e. oxalate has high affinity for Cu complexation (Gamalero *et al*., 2002) forming Cu-oxalate immobile complexes (Ravnskov and Jakobsen, 1999). Thus increasing the availability of Cu in soil by the grown plants could be refereed as a simple mechanism for increasing plant resistance against pathogens especially that Cu salts are widely known for their high ability for controlling the fungi pathogen (Rusjan, 2012). The released oxalates by *S. rolfsii* might sequester calcium from plant cell walls, and thus weaken root cell walls (Jaleel *et al.,* 2007). Therefore the grown plant preserved high concentrations of Cu in the apoplast of plant roots which can rebind root cell walls (Chaignon *et al*., 2002). Concerning the enzymatic responses for *S. rolfsii* invation, pronounced increases in peroxidase activity were detected for plants treated with humic acid exceeding the fungicide treated plants. In this concern, it is thought that plants during pathogen invasion induce the production of reactive oxygen species (O'Brien *et al.,* 2012), which might posses a potential hazard to the pathogen and, at the same time, on the cellular processes of plant/pathogen interactions (Baker and Orlandi, 1995). Then an intermittant step is requried to minimize the effects of the radical oxygen on the grown plant. On the other hand, Zn and Cu were found in high concentrations in plants treated with humic acid. Probably, Zn and Cu stimulated superoxide dismutase turning the superoxide(O^-) into H_2O_2 and thus minimizing the effects of the radical oxygen on the grown plants (Palmer and Guerinot, 2009; Reddi *et al.*, 2009). Afterwards, the activity of peroxydase enzyme was stimulated. Peroxydase enzyme catalyzes oxidation reactions by H_2O_2 through legnin production (Lebeda *et al*., 1999), thus improving the defense mechanisms of plants aginest further pathogen invasion. On the other hand, pronounced increases in the activities of chitinase, peroxidase and polyphenol oxidase enzymes (PPO) were found associated with sulphex treatment. PPO enzymes are among the defense mechanisms that might act againest pathogens (Mayer, 2006), and copper is a structural ion in this enzyme (Tran and Constabel, 2011) with bi-conserved Cu-binding domains i.e. CuA and CuB (Quarta *et al*., 2013). Zn can also stimulate PPO activity (Dongmei *et al*., 2004).

Conclusion:

Humic acid and PBZ could be recommended treatments for increasing the productivity of common beans grown in soil infected with *S. rolfsii* without adverse effects on the environment. Such amendments would increase uptake of N and P and therefore improve the nutritional status of the plants. Increases in Cu and Zn uptake were recorded in the first growing season; however significant reduction in Cu and Zn contents occurred in the different plant parts during the second growing season Such a result probably indicates that Cu and Zn might act as bio-defenders againest the infection by pathogen as they increased in plant tissue during the first growing season of the severe pathogenity as compared with the second growing season with decreasing the severity of the pathogen. It seems that chitinase enzymes in case of PBZ and peroxidase enzymes in case of humic acid were of higher effects as bio-defeners against *S. rolfsii* invasion than the fungicide.

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