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INTERACTION BETWEEN AUXIN & HERBICIDES AND THEIR ROLES IN ROOTING RESPONSE OF FRESH AND AGED MUNG BEAN (*Phaseolus aureus* ROXB.) CUTTINGS.

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Abstract

The influence of auxins and herbicides individually or in combination on rooting response of fresh cuttings, when supplied during ageing, or as inductive auxin treatment (IAT) were studied. The results revealed:

a) *2,4-D at 10^{-3} M or 10^{-4} M for 1/2h as (IAT), had no significant effect on rooting response, when cuttings aged in Paraquat (PQ). Whereas, 2,4-D for 24h at (10^{-4} M) (as IAT) had significant promotion (≥166%) in cuttings aged in PQ. This confirms the role of PQ in stopping the processes that occurs during ageing, but not during rooting per see.*
b) *Replacement of 2,4-D by IAA ($5x10^{-4}$ M) as IAT for 24h doesn't change the trend of events in cuttings aged in PQ or 2,4-D. Seemingly, both herbicides had no effect, when IAT was presented by IAA exclusively.*
c) *Both herbicides had no effect in fresh cutting even when supplied simultaneously whereas, PQ simultaneously with IAA induces 59% over IAA alone.*

Key words: *Ageing, Anti-oxidant defense mechanism, 2,4-D, IAA, Metabolic interaction, Mung bean, Oxidative stress , PQ and Rooting response.*

1- INTRODUCTION

Ageing is a multi-conceptual phenomenon , was studied in different experimental systems and interpreted by several hypotheses , such as, genetic and biochemical changes that lead to the gradual senescence and death of cell or organism(coombs,1986).However in terms of adventitious root formation (ARF), it means the processes that lead to diminishing rooting response in mung bean cuttings by delaying the inductive auxin treatment (cuttings kept in deionized water during ageing period (Jarvis, 1986).

In addition, many attempts have been used to control ageing phenomenon (stopping the processes that occurs during ageing) was archived by several physio – chemical ways . Such as keeping cuttings during ageing period in 1.5% sucrose (Shaheed & Salim ,2002 a), dilute auxins , such as , IAA ,10^{-8}M (Shaheed , 1987) and 400 mg/l as corbate (Shaheed et al, 2010) to maintain the sensitivity of cuttings to inductive auxin treatment (IAT) and inducing a number of roots equal statistically to that in the control (fresh cuttings). Furthermore, using antioxidants of phenolic compounds such as *o*-coummaric acid, caffeic acid, *p*-hydroxy phenol and hydroquinone, all of these at concs. (10^{-3}M (shaheed et, al, 2010). Obviously indole butyric acid (IBA) considered the widely used Auxin in stimulating rooting is due to 1) its ability to stimulate root initiation 2) weak toxicity and persist longer in plant tissues compared to indole acetic acid (IAA) (Hartmann et, al, 2002).

Meanwhile, the insensitivity of olive cuttings var. Domad for exogenously IBA has been interpreted on the basis of differences in metabolism and transport of IBA (Wiesman & epstien, 1987). Whereas, using a combination of IBA and NAA at optimal concentrations increases rooting response in olive

cuttings compared to its supply individually(Isfiendiyaroglu & Ozeker, 2008). Also the ability of some secondary metabolites (growth retardant/ inhibitors) for exp: phenolics, polyamines to modulate the effect of hormones during rooting response (Hartmman et al ,2002).

In addition, hormones and other growth regulators were used for different purposes in the control of some aspects of plants development, and commonly used Auxin in weeds control is 2,4-D (Hopkins & Huner, 2009). The latter indicated that 2,4-D appear as effective Auxin at low conc.s , whereas , it is effective as herbicide at high conc.s . For not clear reasons, these herbicides are toxic, some of these are selectively for broad-leaved like 2,4-D (Chinallia ,2007) and others not selective as (PQ) ,but it is more effective against narrow leaved weeds (Suntres ,2002).

Moreover, the induction of roots formation in cuttings by auxins is still not clear on the molecular level (Ludwig-Muller et, al, 2005). The latter was suggested that rooting response in Arabidopsis cuttings attributed to the interaction between natural Auxin (IAA) and exogenous (IBA). However, the difference in ability for roots formation is due to the difference in metabolism of auxin (Blaskova et, al, 1989) . Obviously Indol butyric acid aspartate (IBASP) is more effective than Free IBA in stimulating adventitious roots formation in mung bean cuttings due to long persistence during rooting (Wiesman, et, al, 1989), As well as other differences in Uptake and Transport which must be taken in a count in rooting pattern (Esptein & Ludwig-Muller, 1993) .

On the other hand, the decline in IAA-Oxidase activity and the high level of auxin protectors may indicate the necessity of auxin accumulation in the induction of rooting as confirmed by several previous studies, such as. (Liu et, al, 1996) .While period that coincides with the highest enzyme activity and the lowest level for the auxin protectors linked to the early initiation phase, they may indicate requirements to reduce the normal level (IAA) for an appropriate hormonal balance (Moncousin, 1991). At the level of physiological events that lead to initiation of roots, Ludwig-Muller and his colleaques (2005) was found that: 1) IBA is one of the natural factors in Arabidopsis (but not IAA) induce adventitious root formation. 2) The timing of Auxin supply is important to distinguish between Callus and roots formation. 3) The system used (cuttings from Arabidopsis) is appropriate for the diagnosis of genes that correlated with root formation. 4) as well as the IAA-deficient Mutant has been used to analyze the overlap between the IBA / IAA during root formation. Moreover , research lines have shown that part of IBA effect was attributed to direct action of IBA as normal auxin (Poupart & Waddell, 2000), despite the possibility of converting IBA to IAA through β - Oxidation (Bartel , et al; 2001). For example , the drought stress and osmotic stress stimulants synthesis of IBA and thereby increase its content, while IAA is less affected (Ludwig-Muller et al; 1995).

Finally, it is common in agricultural practices, that cuttings were treated with herbicides due to the possibility that cuttings may be infected by various fungi during rooting (Thielges & Hoitink, 1972), a condition that those herbicides does not affect rooting response positively or negatively in case of induction of these cuttings by a particular chemical. Given that lack of a study on the already interaction of herbicides and Auxin in rooting response of fresh and aged cuttings of mung bean, as well as keeping cuttings during ageing period as inductive auxin treatment after ageing was considered the aim of this study.

2- MATERIALS AND METHODS

2-1: Growth of stock plants & Preparation of cuttings :-

Seeds (*Phaseolus aureus* Roxb.Var.local) germination and seedlings growth were carried out in growth cabinet at $25_{\pm}1C^{\circ}$ under continuous illumination supplied by warm white fluorescent tubes

(1500-1800 lux) and relative humidity of 60 -70% (Binder GMbH/ Germany). Stem cuttings were prepared according to (Hess, 1961) from 10- day – old light grown seedlings. The cutting had apical bud, a pair of fully expanded primary leaves , epicotyl and 3cm of hypocotyl under cotyledonary nodes , after removal of root system .

2-2: Preparation of solutions

2,4-D and IAA were initially dissolved in small volume of absolute ethanol (Final conc. of abs . eth . is 0.2%) . This conc. is not effective in ARF of mung been cuttings (Middleton et al ; 1978 a). PQ (27% active substance / United phosphorus Ltd India) was prepared at 1% (v/v) in d/H2O , then diluted to the required concentrations.

2-3: Basal treatment of cuttings

Basal part (hypocotyl) of fresh cuttings were treated for 1/2h or 24h with tested solutions (d/H2O, 2,4-D or IAA) or in combination of these solutions . Thereafter ,cuttings were transferred to boric acid (5µg /ml) for 6 days as rooting medium because of the role of boron in development of root primordial in to visible roots (Middleton et al; 1978 b).

2-4: Ageing treatments

Cuttings were aged by dipping in d/H2O or in appropriate tested solutions of PQ an 2,4-D of different conc.s for 3-days prior to their treatment with IAA or alternatively with 2,4-D (as inductive auxin treatment = IAT). During all these treatments, cuttings were held under the same conditions as mentioned above for raising stock seedlings.

Twelve cuttings were used per treatment for rooting test were placed 4 per glass vial containing 15ml (3cm depth) of the appropriate solution . All experiments were designed as completes randomized design for statistical analysis , depending on L.S.D and T-test for treatment comparison by using analysis of variance (ANOVA) for all experiments as individual (Spiegel , 1975).

3- RESULTS

The effect of 2,4-D on rooting response of cuttings already aged for 3-days in different conc. of PQ was revealed in table (1) . The average roots number of cuttings aged in d/H2O for 3 – days and in the absence of supplied auxin (general control) is 6.3 roots/cutting. whereas , cuttings aged in d/H2O for 3-days before supplying the inductive auxin treatment (IAT) (2,4-D) at 10^{-3}M for 1/2 h were developed 22.3 roots . Which is significantly effective by 3.5 folds compared to control (253.9 %) . However ,cuttings were aged in PQ particularly at conc. (1×10^{-7}%), prior to exogenous supply of 2,4-D , (10^{-3}M) (IAT) developed 26 roots /cutting , which is not significant compared to 2,4-D alone (cuttings were kept in d/H2O during ageing period instead of PQ thereafter , supplied with 2,4-D as IAT (22.3 roots) .

For controlling the processes that occurred during ageing in cuttings aged in different conc.s of PQ, the above experiment has been repeated twice: a) with a lower conc. of 2,4-D (10^{-4}M) as IAT & at the same period (1/2h). b) with a lower conc. (10^{-4}M) of 2,4-D as IAT but for 24h. The results of 1st experiment (Table-2) were reveled that cuttings already aged in PQ at conc. (1×10^{-7} %) were maintained its sensitivity for IAT represented by 2,4-D , that developed a higher number of roots equal to 18. The latter figure statistically represent a significant increase (146.5%)on 0.01 level of probability compared to cuttings aged in d/H2O & untreated with auxin (control) whereas , such increase is not significant in comparison to cuttings supplied with 2,4-D (10^{-4} M). In other words, PQ does not stop the processes that occurs during ageing at this conc. Mean-while, other conc. have no significant effect on rooting response except the inhibitory conc. (1×10^{-8}%) at 0.05 level .

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Table(1): Effect of 2,4-D, 10^{-3} M for 1/2h on rooting response of mung bean cuttings aged in paraquat

Cuttings aged for 3-days in :-	Subsequent treatment for 1/2h :-	Mean root number / cutting
d/H2O	d/H2O	6.3
d/H2O	2,4-D , 10^{-3}M	22.3
PQ , (5 X 10^{-7})%	2,4-D , 10^{-3}M	20
PQ , (1 X 10^{-7})%	2,4-D , 10^{-3}M	26*
PQ , (5 X 10^{-8})%	2,4-D , 10^{-3}M	21
PQ , (1 X 10^{-8})%	2,4-D , 10^{-3}M	11.6

*L .S. D (0.05) = 8.874 ; L. S . D. (0.01) = 11.951.

Table(2):Effect of 2,4-D ,10^{-4} M for 1/2h on rooting response of mung bean cuttings aged in paraquat

Cuttings aged for 3-days in :-	Subsequent treatment for 1/2h :-	Mean root number / cutting
d/H2O	d/H2O	7.3
d/H2O	2,4-D , 10^{-4}M	17.3
PQ , (5 X 10^{-7})%	2,4-D , 10^{-4}M	12.6
PQ , (1 X 10^{-7})%	2,4-D , 10^{-4}M	18*
PQ , (5 X 10^{-8})%	2,4-D , 10^{-4}M	16
PQ , (1 X 10^{-8})%	2,4-D , 10^{-4}M	9.3

*L .S. D (0.05) = 5.476 ; L. S . D. (0.01) = 7.375.

The results of 2nd experiment (Table-3) were revealed that PQ at conc. (1×10^{-7}%) stopped all processes that occurs in cuttings aged in PQ , that developed 31 roots with increment equal to 166.3% compared to cutting aged in d/H2O & consequently supplied with 2,4-D as IAT (19.3 roots) . The latter case, confirm the role of PQ in stopping the processes that occurs during ageing *per sue*(but not during rooting response) particularly when 2,4-D was supplied for 24h as IAT.

Table (3): Effect of 2,4-D, 10^{-4} M for 24h on rooting response of mung bean cuttings aged in paraquat

Cuttings aged for 3-days in	Subsequent treatment for 24h :-	Mean root number / cutting
d/H2O	d/H2O	8.3
d/H2O	2,4-D , 10^{-4}M	19.3
PQ , (5 X 10^{-7})%	2,4-D , 10^{-4}M	22
PQ , (1 X 10^{-7})%	2,4-D , 10^{-4}M	31*
PQ , (5 X 10^{-8})%	2,4-D , 10^{-4}M	24
PQ , (1 X 10^{-8})%	2,4-D , 10^{-4}M	15.3

*L .S. D (0.05) = 9.621 ; L. S . D. (0.01) = 12.957.

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Table (4) shows that rooting response of mung bean cuttings were aged in different conc. of PQ and induced by IAA . The results were revealed that fresh untreated cuttings were developed (13) roots. This was attributed to the endogenous IAA whereas , fresh cuttings treated with IAA , 5×10^{-4} M for 24h were developed 72.8 roots (increasing % is 460). However , in case of cuttings aged in d/H2O for 3-days then transferred to boric acid (5 µg/ml) as rooting medium without supplying IAT were developed 9.2 roots , which represent a decline % in rooting response equal to 29.2 % compared to control (fresh cuttings = 13 roots) .

On the other hand , when IAT was delayed , by ageing the cuttings in d/H2O for an intervening period (3-day), rooting response progressively declined to 46.4 roots (inhibition %=36.2) compared to fresh induced cuttings . Statistically this percent of diminishing rooting response is highly significant & attributed to the processes that occurs during ageing .

For controlling these processes (delaying) , cutting were kept for 3-days in different concs. of PQ. Generally cuttings aged in all tested conc. of PQ were developed a number of roots restricted between (34.1 -48.6 root/ cutting)after supplying IAA as IAT . The rooting response of these cuttings represented by such figures are not significant compared to cuttings aged in d/H2O (46.4 roots). The foregoing results confirm that PQ has no effect on processes that occurs during ageing particularly when IAA was supplied for 24h on IAT.

Table(4): Effect of IAA, 5×10^{-4} for 24h on rooting response of mung bean cuttings aged in paraquat .

Cuttings aged for 3-days in :-	Subsequent treatment for 24h	Mean root number / cutting
-	d/H2O	13
-	10^{-4} , M× IAA,5	72.8
d/H2O	d/H2O	9.2
d/H2O	10^{-4} , M× IAA,5	46.4
PQ , (5 X 10^{-7})%	10^{-4} , M× IAA,5	40.1
PQ , (5 X 10^{-7})%	10^{-4} , M× IAA,5	48.6*
PQ , (5 X 10^{-7})%	10^{-4} , M× IAA,5	35.3
PQ , (5 X 10^{-7})%	10^{-4} , M× IAA,5	34.1
PQ , (5 X 10^{-7})%	10^{-4} , M× IAA,5	34.7
PQ , (5 X 10^{-7})%	10^{-4} , M× IAA,5	44.3

*L .S. D (0.05) = 17.879 ; L. S . D. (0.01) = 23.780 .

In comparison with PQ , 2,4-D was supplied at different conc. during ageing of cuttings for 3-days prior to supply IAA , 5×10^{-4} M as IAT (Table-5) . Generally , cuttings aged in all tested conc.s Of 2,4-D (10^{-8} -10^{-4} M) were developed (32.6-50.8 roots/ cutting) after supplying IAA as IAT . These figures had no significancy compared to control treatment , as it was the case with PQ . Obviously , these results confirms that 2,4-D had no effect too, on processes that occurs during ageing .

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Table (5): Effect of IAA ,5 × 10^{-4} M for 24h on rooting response of mung bean cuttings aged in 2,4-D.

Cuttings aged for 3-days in :-	Subsequent treatment for 24h :-	Mean root number / cutting
-	d/H2O	13
-	10^{-4} , M× IAA ,5	72.8
d/H2O	d/H2O	9.2
d/H2O	10^{-4} , M× IAA ,5	46.4
2,4-D , 10^{-4}M	10^{-4} , M× IAA ,5	39.6
2,4-D , 10^{-5}M	10^{-4} , M× IAA ,5	32.7
2,4-D , 10^{-6}M	10^{-4} , M× IAA ,5	42.9
2,4-D , 10^{-7}M	10^{-4} , M× IAA ,5	44.1
2,4-D , 10^{-8}M	10^{-4} , M× IAA ,5	50.8*

*L .S. D (0.05) = 13 ; L. S . D. (0.01) = 17.293 .

Interaction between 2,4-D &PQ in rooting response of fresh mung bean cuttings has, been shown in table (6) .Fresh cuttings were treated with optimal conc. of 2,4-D were developed (31.6 roots) compared to fresh untreated cuttings (8.6 roots) . However , cuttings were supplied with different combinations of 2,4-D & PQ for 1/2 h, have no significant promotory effect compared to control (2,4-D) ,except the combination [2,4-D, 10^{-3}M + PQ , 5×10^{-6}%] that developed the highest number 22.3 roots, with increasing % equal to 159% compared to control (d/H2O).

Table (6): Effect of 2,4-D & PQ supplied simultaneously in combination on rooting response of fresh mung bean cuttings.

Treatment for 24h :-	Mean root number / cutting
d/H2O	8.6
2,4-D , 10^{-3}M	31.6
2,4-D , 10^{-3}M + PQ , (5 X 10^{-6}) %	22.3*
2,4-D , 10^{-3}M + PQ , (5 X 10^{-6}) %	12.3
2,4-D , 10^{-4}M + PQ , (5 X 10^{-6}) %	1.8
2,4-D , 10^{-4}M + PQ , (5 X 10^{-6}) %	7.3

*L .S. D (0.05) = 9.617 ; L. S . D. (0.01) = 12.979 .

On the other hand , the interaction between IAA &PQ in rooting response of fresh mung bean cuttings has been shown in table (7). Obviously , IAA at optimal conc. 5×10^{-4} M developed 45.3 root/cutting compared with fresh untreated cutting (8.6 roots) . However ,combination between IAA 5×10^{-4} M & PQ at two conc.(1×10^{-6} % & 5×10^{-6} %) induce 61.8 & 72.1 roots respectively . It is noteworthy , that interaction between auxins particularly IAA & herbicides particularly PQ was induced adventitious root formation in fresh mung bean cuttings which is 59% over IAA alone .

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Table(7):Effect of IAA & PQ supplied simultaneously in combination on rooting response of fresh mung bean cuttings.

Treatment for 24h :-	Mean root number / cutting
d/H2O	8.6
10^{-4} M × IAA ,5	45.3
10^{-4} M × IAA ,5 + PQ , (5 X 10^{-6}) %	72.1*
10^{-4} M × IAA ,5 + PQ , (5 X 10^{-6}) %	61.8

*L .S. D (0.05) = 19.035 ; L. S . D. (0.01) = 25.916 .

4- DISCUSSION

Fresh cuttings were induced by (IAA,5×10^{-4}M) as IAT immediately when taken from 10-day old light grown seedling were developed 72.8 roots/cuttings (tables 5&4) . Whereas, any delay in supplying of IAT by keeping cuttings in d/H2O for 3-day caused decline in rooting response by 36.3%. The above decline was considered as physiological signal for biochemical processes that occurs during ageing and lead to diminishing rooting response (shaheed,1987,Shaheed & Jabar,2009). General knowledge was pointed out 10-hypothethses that explain ageing causes in different experimental systems , which are mostly denoted the decline in rooting response , as a result of dealing auxin supply by keeping cutting in d/ H2O for 3- days.

The results of the current study revealed that cuttings aged in d/H2O when supplied with 2,4-D for 1/2 h at 10^{-3} M (the optimal conc. When supplied at the optimal duration for fresh cuttings , data not presented) as IAT, developed 22.3 roots/ cutting , compared to cuttings aged in d/H2O but not induced by auxin (2,4-D) that developed 6.3 roots (Table 1) . This is occurring with other synthetic auxins that induce rooting such as IAA, IBA, NAA, so it was agreed with Grabinske and his colleagues (2003) about 2,4-D that acting as herbicide at high conc. from one side whereas , acts as endogenous plant hormones (IAA) at low conc. from other side . In addition , that all auxins can acts in the same trend by manipulating the kind of cuttings , kind of auxin , auxin conc., duration of auxin treatment , or duration between cutting preparation from stock plant & auxin treatment (ageing period) (Jarvis,1986) For controlling the processes that occurred during ageing phenomenon , cuttings were kept at different conc.s of herbicide PQ as alternative for d/ H2O . Clearly, PQ has no significant effect on these processes whether 2,4-D was supplied as IAT (after ageing treatment for 3-days) for 1/2 h. & at conc. 10^{-3} M (Table -1) or at the same duration (1/2h) but with lower conc. 10^{-4} M (Table- 2) . Consequentially , PQ has no effect at low conc. Whereas at (1×10^{-7}%) when 2,4-D was supplied as IAT for 24h (Table -3),that developed a higher average of roots 31 with an statistically significant increase of about 166% compared to cuttings aged in d/H2O and induced by 2,4 –D (Table -3).

Obviously the latter case, raises that lowering conc. of 2,4-D (from 10^{-3} -10^{-4} M) was coincided with the prolonging the duration of treatment (from 1/2h- 24h), have remarkable effect in increasing the rooting response of mung bean cuttings aged in PQ . This may be attributed to the interaction between /or action of 2,4-D with metabolic pathways of PQ (Table-6). Meanwhile, confirming the role of PQ in stopping the processes (partialy/ completely according to the sp.) that occurs during ageing *per sue* , but not during rooting .

On the other hand , replacement of 2,4-D by IAA (5×10^{-4} M) as IAT for 24h doesn't change the trend of events in cuttings aged in different conc. of PQ (Table -4) or 2,4-D (Table -5) . In other words ,

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both herbicides have no effect on rooting processes particularly , when IAT was presented by IAA exclusively . Seemingly , the effect of PQ through the processes that occurs during ageing of mung bean cuttings is (only positive and significant when IAT was presented as IAA.

To verify the role of PQ in ageing processes or rooting processes via interaction between herbicides & auxins using fresh cuttings , the results were revealed that both herbicides (PQ / 2,4-D) has no significantly positive effect on rooting response ,even when supplied simultaneously at optimal conc. for both (22.3 roots/table- 6) compared to 2,4-D alone (31.6 roots) whereas , supplying PQ simultaneously with IAA, significantly (on 0.01 level of probability) promotes rooting response with increment equal 59.1% over IAA alone (table-7) . According to the forgoing results, PQ is effective in aged cuttings when 2,4-D supplied as IAT. Meanwhile, PQ is effective too in fresh cuttings when supplied simultaneously only with IAA . However , as a conclusion , PQ acts synergistically with IAA in fresh cuttings but nut with 2,4-D whereas , PQ acts synergistically too with 2,4-D as IAT in aged cutting but not with IAA. It is difficult to give plausible explanation for the above conclusion . However , the 1st case that involve the synergestic effect of PQ with IAA in fresh cuttings ,may be attributed to the role of PQ in stimulation of IAA-oxidase . The latter is acting for diminishing IAA to the level that required for growth & development of root primordia to visible roots (chibbar et al,979), after its initiation under the influence of IAA at the 1st phase .

Consequently , the 2nd case that involve the synergistic effect of PQ with 2,4-d in aged cuttings , may be attributed to the role of PQ in preventing suberin bioth *Babylon University,* entheses promoting or suberin hydrolysis (loosing) after its biosyntheses .this is in agreement with Clin&Neely (1983),that hypothesis that explain ageing causes (blockage of xylem vessels by subrerin during ageing). This hypothesis was verified physiologically &anatomically in terms of rooting response of mung bean cuttings by (shaheed & Al-alwani, 2002) . the later conforming the above explanation by maintaining the translocation elements of xylem in active state functionally via herbicides transport (David ,1996) . seemingly , the plant tissue maintaining their integrity morphologically , through the action of herbicides on suberin loosening that lining xylem vessels org filling it with progression of ageing period.

Alternatively , the decline of SOD & CAT activity in different parts (hypocotyl , epicotyl and primary leaves) of mung bean cuttings aged in d/H2O (Shaheed et al 2010),give clear imagination about oxidative processes that occurs during ageing (Harman, 1956 ; shaheed et al ,2009 a) , its role in degrading the biological molecules & some cellular organelles that reduce the biosynthesis of rooting response pre requisites (Scandalios ,1993) .

Such imagination may occurs via regeneration of free radicals, which by somehow influence the pathways of hormone biosynthesis , in particular that have the priority in rooting response (as auxin) . So, accordingly an increase in the activity of SOD ,CAT enzymes may abstract the way against ROS to react with the important molecules through increase the enzymatic activity of anti-oxidant defense mechanism represented by SOD /CAT in mung bean cuttings aged in 2,4-D at 10^{-3} M for 1/2 h. (Al-alwani ،et al, 2012) .

Notwithstanding ,the latter case agreed with shaaltiel and Gresssel (1986) about the role of PQ in increasing the activity of ant-oxidant defense enzyme such as SOD/CAT in *Conyza bonariensis*, because that herbicides considered as one of the factors causing the oxidative stress (scandalios, 1993). So the produced ROS (e.g. superoxide *per sue*) that converted into H2O2 in the presence of SOD. Consequently, H2O2 should be removed by catalases (peroxides) (Suntres ,2002). In other words, the resistant was correlated with increasing SOD activity and other enzymes that capable for scavenging the regenerated free radicals .

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EFFECT OF CONCENTRATION AND APPLICATION METHOD OF GA_3, SALICYLIC AND ASCORBIC ACIDS ON SEED YIED OF BROAD BEAN (Vicia faba L.) UNDER SALINE SOIL

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Abstract

An experiment was conducted at Al-Kefel, Babylon, to study the effect of seven acid treatments (GA_3, 50 and 100 mg/l ; Salicylic, 0.5 and 1 mM ; Ascorbic, 50 and 100 mg/l, in addition to the control treatment with water) and its interactions with three application methods (seed priming, foliar, priming+ foliar) on yield parameters of broad bean plants under saline soil (7.5 dsm^{-1}) in 2011-2012 growth season. Randomized complete block design with three replications was used. The results showed as follow: Treatment with GA_3 (100 mg/l) or salicylic acid (1 mM) alleviated salt damage and caused a significant increase in pod numbers/pant (10.6 and 9.6), green pod weight (20.8 and 20.2 gm), weight of 100 seeds (128.2 and 132.2 gm), green pods yield (12.22 and 11.9 ton/hectare) and dry seed yield (3.44 and 3.27 ton/hectare) as compared to control.

Key world: b*road bean, saline soil, GA_3, salicylic acid, ascorbic acid, foliar application*

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INTRODUCTION

Broad bean (Vicia faba L.) is an important legume crops grown for its wet and dry seed. And it is cultivated in crop rotations to improve soil properties (Jassim, 2007). Farmers accept planted broad bean because its low cost cultivation and ease of transfer and circulation.

Salinity is major harmful environmental factor that poses a major threat to crop productivity mainly in arid and semiarid region of the world (Munns, 2005). Effects of salinity are more obvious in arid and semiarid regions where limited rainfall, high evaporate transpiration, and high temperature associated with poor water and soil management practices are the major contributing factors (Azevedo Neto et al., 2006). Plant productivity is severely threatened by salinity. About 800 million hectares of land throughout the world are salt-affected (Munns, 2005). Salinity is due to high amount of soluble salts in soils or waters. Elevated levels of soluble salts in the soils as well as water result in restricted growth and yield production in arid and semi-arid regions (Flowers et al., 2010). It has been well practiced that when electrical conductivity (E.C.) exceeds greater than 4 $ds.m^{-1}$, a sharp decrease in the crop yield has been observed, but the level of reduction depends upon the tolerance potential of crop plants (Sairam et al., 2002). The threshold of broad bean is 1.6 $ds.m^{-1}$ and the decrease in yield is 9.6% in each increasing 1 $ds.m^{-1}$ (Ayers and Eberhard, 1960). Photosynthesis, decreases due to salt-induced destruction of photosynthesizing tissues, reduced water potential and high accumulation of Na^+ and Cl^- (Ashraf et al., 2008). The adverse effects of salt stress on photosynthesis as well as plant growth are correlated with oxidative stress induced by generation of excessive amounts of ROS and imbalance in regulation of antioxidative defensive mechanism (Noreen et al., 2010).

GAs are phytohormons known to induce various physiological responses in plants and alter plant metabolism under stress. GA_3 increase photosynthetic rate in leaves of bean(Khan, 2002). GA_3 is important in influencing the photosynthetic activity and consequently the source–sink relations. The

increase in glucose content by GA_3 application shows the efficiency of GA_3 in increasing photosynthesis (Brian et al., 2006).

Salicylic acid (SA) is an endogenous growth regulator, which participates in the regulation of physiological processes in plants. SA mediates the oxidative burst that leads to cell death in the hypersensitive response and acts as a signal for the development of systemic acquired resistance (Shirasu et al., 1997). SA had a major role in modulating the plant response to several abiotic stresses (Senaratna et al., 2000). SA has been reported to ameliorate the adverse effects of salinity by improving the functioning of the photosynthetic pigments and antioxidative enzymes (Idrees et al,. 2010; 2011). SA is also considered to be a signaling molecule that plays a key role in growth, development, and defense responses in plants under stress conditions (Cameron, 2000).

Ascorbic acid (AsA) is one of the most important antioxidants abundantly occurring in plants (Smirnoff, 2000). High endogenous AsA in plants is necessary to counteract oxidative stress in addition to regulating other processes of plant metabolism. Endogenous AsA can be increased by exogenous application of AsA through the rooting medium (Chen and Gallie, 2004), as a foliar spray or as seed priming.

Studies showed that application methods affecting the efficiency of growth regulators. Unamba and others (2009) added GA_3 on okra by priming or spraying at low-lying (0-30 mg/l), and reported that both methods were effective, but spraying was the most efficient way than priming in its effect. Akbari and others (2008) added GA_3 by priming , spraying or priming+ spraying at saline stress, they found that priming+ spray was the best in mitigate salt damage and gave the best results.

Nevertheless, there have been no quantitative investigations of the effects of an additional supply of GA_3, salicylic acid and ascorbic acid on broad bean. The objectives of this study were to determine how GA_3, salicylic acid and ascorbic acid affect yield of broad bean under salt stress.

MATERIALS AND METHODS

Experiment was conducted in a private farm in the Al-Kefel area, Babylon governorate/Iraq during 2011-2012 growth season to study the effect of seven treatments: GA_3 at 50 and 100 mg/l, Salicylic Acid (SA) at 50 and 100 mM, Ascorbic Acid (AsA) at 50 and 100 mg/l in addition to water treatment (as control), and its interactions with three application methods: priming, spraying, priming+ spraying, on yield of broad bean plants under salt stress.

Soil was chosen at E.C. 7.5 dsm^{-1}. Complete Randomized Block Design with three replications was used. The seeds were priming for 24 hours according to treatments and seeded in 14/10/2011 in rows 75 cm width , the distance between hills along the row was 20 cm. Plot area was 6.75 m^2(3 rows with 4 meters long). Crop and soil service were conducted as recommended in the cultivation of broad bean. Foliar application was carried out twice , the first was done after one month from emergence and the second after one month later the first spray. Average of pods number/plant, seeds number per pods, green pod yield(t/ha) at seed filling stage (from one row of each experimental unit) , 100 dry seeds weight and dry seed yield (t/ha) from the other two rows of each experimental unit , were determined. Statistical analyses were performed with SPSS packet software and the values of least significant differences ($LSD_{0.05}$) were calculated to compare the means of different treatments.

RESULTD AND DISCUSSION

Table (1) shows that there is significant superiority for GA_3 100 mg/l compared to control. It was attributed to the increase in vegetative growth (Ali and Matar, $2013_{a,b}$). Salinity causes premature

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senescence which reduce the amount of assimilate that the pant can produce and a reduction in the assimilate transported to the growing tissue may further limit growth (Munns, 2002) which produce least pod number and weight. Addition of GA_3 will increase the pod number and this was confirmed with (Shah, 2007) in his study on mustard plant when sprayed with 10^{-5} mole of GA_3 caused a significant increase in dry weight , leaf area , chlorophyll content and the rate of photosynthesis which led to an increase in pod number. (Alonso-Ramirez et al., 2009) reported that GA_3 by repressing Della proteins and changing the SA/JA balance are able to control plant responses to stress. SA had no significant superiority to the rest of the transactions (except the GA_3 100 mg/l) and this attributed to minimize the falling and abortion flowers and keep flowers from drought and encourage pollination flowers internally before blooming, (Amanullah et al, 2010). And also to its role in the inhibition of ethylene, ABA and enzyme pectin methyl esterase which lead to encourage pod falling(Gupta, 2011). The harmful effects of salinity will alleviate by SA treatment from the possibility of renew sink and improve the rate of accumulation of dry mass in the development of developing productive organs(Davies, 1995). SA contributes in increasing the efficiency and the use of water in addition to protecting pigments and photosynthesis device (Hassanein, 2009). This results agreed with the finding of (Azooz, 2009) on faba bean. The table showed that application methods and the interaction between treatments had no significant effect on plant pod numbers.

Table (1): Effect of GA3, SA and AsA, and its interactions with application methods on pod numbers in plant

B / A	water	GA_3 50mg/l	GA_3 100mg/l	SA 0.5mM	SA 1mM	AsA 100mg/l	AsA 50mg/l	Mean A
soaking	9.3	10.0	10.0	9.3	10. 7	9.3	9. 7	9.8
spraying	9.0	9. 7	10. 7	9.0	11.0	10.0	10.0	9.9
Soaking+ spraying	10.0	10. 7	11.0	10. 3	9.7	9.0	9. 7	10.5
Mean B	9.4	10.1	10. 6	9. 6	10.4	9.4	9. 8	
$LSD_{0.05}$	LSD A= n.s				LSD B=1.179			LSD AB= n.s

Table (2) Showed that GA_3 100mg/l and SA 1mM treatments were superior and significantly increased average weight of green pod compared to control and the percentage of increases were (8.12 to 2.16%), respectively. It has been proposed that under salt stress conditions the thickness of the assimilate conducting pathway is reduced (Aldesuquy and Ibrahim, 2001), and leaves start behaving as sinks rather than sources (Arbona et al., 2005). This causes inhibition of assimilate movement towards the developing reproductive organs, which might be the reason for the observed decrease in pod number, seeds per pod, 1000-seed weight and seed yield per plant. On the other hand, these adverse effects of high salinity were alleviated by the hormone treatment, primarily by rejuvenation of the sink potential and enhancement of the duration or rate of dry mass accumulation in developing reproductive organs (Davies, 1995). GA_3 is known to have a secondary enhancement effect on protein content through the intensification of nitrate reductase activity (Shah, 2004). Stimulation of the

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enzyme protein synthesis by GA_3 stimulates the overall protein synthesis (Premabatidevi, 1998). It indicate that the ability of GA_3 to ameliorate all adverse effects of salt stress and rescue the pod weight productivity. SA treatment increased the weight of green pod and it was due to increased in chlorophyll content, modify the content of nutrients elements and reduce the toxic ions content in plant leaves (Ali and Matar, $2013_{a,b}$). SA role in increasing the proportion of cytokines and increase the proportion of cell divisions and the number of cells (Sanna et al., 2001). These results are consistent with (Kumar et al., 2000) on soybean and (Zaghlool, 2002) on mung bean. On the other hand AsA had no significant effect, but tended to increase average pod weight. The table showed that method of applications and the interaction had no significant effect on the weight of green pod.

Table (2): Effect of GA3, SA and AsA, and its interactions with application methods on green pod weight (gm)

B / A	water	GA_3 50mg/l	GA_3 100mg/l	SA 0.5mM	SA 1mM	AsA 100mg/l	AsA 50mg/l	Mean A
soaking	18.0	18.1	20.7	18.17	19.8	17.6	19.2	18.8
spraying	17.7	17.9	20.4	17.9	20.3	17.8	18.6	18. 7
Soaking+ spraying	18.1	19.8	21.3	19.9	20.5	18.8	18.7	19. 6
Mean B	17.9	18.6	20.8	18. 7	20.2	18.1	18.8	
$LSD_{0.05}$	LSD A=n.s				LSD B= 1.38			LSD AB= n.s.

Table (3) shows that GA_3 at 50 and 100 mg/l and SA at 0.5 and 1 mM were superior significantly on increasing weight of 100 seeds compared to control. The increases by GA_3 due to its ability to ameliorate all adverse effects of salt stress. Or as a result that GA_3 stimulate to build nucleic acids DNA and RNA, thereby increasing protein building and biological processes within the plant (Al-Asadi, 2006). Under stress conditions beards, the leaves begin to behave like a sink rather than asource. And that these reasons reduce the representation movement towards developing and producing organs(Arbona et al., 2005), that are a major cause of the decline is noticeable in green pod weight. This results was agreed with (Sarkar et al., 2002) who found that 100 seed weight increase in soybean plants with adding GA3 100 mg/l. On the other hand, SA led to alleviate salinity damage through the ability to link with amino acids and its role in increasing the production of nucleic acids RNA, DNA in addition. AsA had no significant effect , but tended to mitigation of salt stress damage. The table showed that methods of application and the interaction had no significant effect.

Table(4) showed that GA_3 100 mg/l and SA 1mM caused a significant increase in green pods yield compared to control, with an increase of 19.6 and 16.5% respectively. The increase due to help the plant to ameliorate the slat stress conditions by improving vegetative growth, pigments content, modify the content of nutrients elements and reduce leaves content of toxic ions and reduce oxidative damage (Jasim and Matar, $2013_{a,b}$). it caused increases in photosynthetic potential of plant resulting in more photosynthate production which in turn enhances the sink strength(Iqbal et al., 2011). On the other hand SA alleviate the harmful effect of salt stress (El-Khallal et al., 2009). This results agreed

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with (Turkyilmaz et al., 2005) on bean and (Jeyakumar et al., 2008) on mung bean. AsA had no significant effect. AsA had no significant effect. The table showed that application methods and the interaction had no significant effect on green pod yield.

Table (3): Effect of GA3, SA and AsA, and its interactions with application methods on 100 seeds weight (gm)

B / A	water	GA_3 50mg/l	GA_3 100mg/l	SA 0.5mM	SA 1mM	AsA 100mg/l	AsA 50mg/l	Mean A
priming	119.9	124.8	129.6	126.5	132.1	124.0	124.9	126.0
spraying	122.3	127.7	130.0	127.1	131.6	121.8	124.1	126.4
priming+ spraying	120.0	132.1	129.3	131.4	133.1	125. 6	122.1	127.6
Mean B	120.7	128.2	129.6	128.3	132.2	123. 8	123.7	
$LSD_{0.05}$	LSD A= n.s				LSD B= 7.17			LSD AB= n.s

Table (4) : Effect of GA3, SA and AsA, and its interactions with application methods on green pod yield (ton/ha)

B / A	water	GA_3 50mg/l	GA_3 100mg/l	SA 0.5mM	SA 1mM	AsA 100mg/l	AsA 50mg/l	Mean A
Soaking	10.072	10.901	12.404	10.133	11.831	9.798	11.103	10. 984
Spraying	9.701	10.373	11.971	9.973	12.034	10.601	11.132	10.826
Soaking+ spraying	10.803	12.030	12.304	11.630	11.832	10.074	10.733	11.344
Mean B	10.202	11.101	12.223	10.582	11.902	10.161	10.992	
$LSD_{0.05}$	LSD A= n.s.				LSD B= 1.133			LSD AB = n.s.

Table(5) showed that GA_3 , SA at all concentrations caused a significant increase in dry seed yield compared to control. GA_3 100 mg/l was superior significantly compared to all treatment (except SA 1mM). This increase was due to increases in chlorophyll and vegetative growth, and reduce leaf content of the toxic ions and increasing leaf content of potassium and calcium (Jasim and Matar, $2013_{a,b}$). The photoassimilates produced under salt stress are used to support crucial processes such as growth and osmotic adjustment. In general, salinity causes a reduction in sink enzyme activities, leading to an increase in sucrose in source leaves, with a decrease in photosynthesis rate by feedback inhibition (Poljakoff-Mayber and Lerner, 1994). Application of GA is found to induce extracellular

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invertase, which acts on sucrose to form hexose. The hexose formed is easily transported to the sink, thereby increasing sink strength. The induction of extracellular invertase by both abiotic and biotic stress stimuli supports the suggestion that extracellular invertase is not only a key modulator of assimilate partitioning, but is also an important component of various stress responses. Similarly, GA increases photosynthetic rate under stress and thus enhances the source potential (Iqbal et al., 2011). These results were agreed with (EL-Shraiy and Hegazi, 2009) on peas and (Gherroucha et al., 2011) on wheat. AsA had no significant effect, but it tend to increase. The table showed that methods of application and the interaction had no significant effect.

Table (5): Effect of GA3, SA and AsA, and its interactions with application methods on dry seed yield (ton/ha)

B / A	water	GA_3 50mg/l	GA_3 100mg/l	SA 0.5mM	SA 1mM	AsA 50mg/l	AsA 100mg/l	Mean A
Soaking	2.714	2.932	3.352	2.924	3.421	2.897	2.976	3.031
Spraying	2.573	2.808	3.481	2.804	3.210	2.873	2.797	2.935
Soaking+ spraying	2.554	3.314	3.514	3.273	3.199	2.889	2.996	3.106
Mean of B	2.614	3.018	3.449	3.000	3.273	2.886	2.926	
LSD0.05	LSD A= n.s.			LSD B= 0.539			LSD AB = 0.313	

CONCLUSION

We concluded that treatment with 100 mg GA3/l and 1MM SA led to mitigate the damage of salt stress on bean yield, while ASA had no significant effect, but tend to minimize the damage of salt stress. The application of methods and interactions between treatments had no significant effect. We recommend conducting subsequent studies inflict by using higher concentrations and other crops.

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SOME PHYSIOLOGICAL PARAMETERS AS SCREENING TOOLS FOR DROUGHT TOLERANCE IN BREAD WHEAT LINES (TRITICUM AESTIVAM L.)

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Abstract

Two greenhouse experiments were carried out at the Institute of Biological Production Systems, Leibniz Universität Hannover, Germany during 2008/2009 and 2009/2010 growing seasons to study the influence of the osmotic adjustment (OA) capacity, relative water content (RWC) and specific leaf area (SLA) on tolerance to drought in 22 breeding lines, two parents and tolerant cultivar (Sahel 1) of bread wheat (Triticum aestivam L.) under drought conditions. Differences were seen in of the OA, RWC and SLA of the different genotypes. Four of the breeding lines showed the greatest osmotic adjustment capacities, high RWC and good SLA values under drought stress conditions better than the tolerant cultivar. The heritability of OA, RWC and SLA was 0.56, 0.49 and 0.88, respectively. The results indicated that osmotic adjustment, as well as RWC and SLA could be used as screening tools for drought resistant bread wheat genotypes in the greenhouse. This study also demonstrated the appropriate greenhouse screening methodology in this regard.

Key words: *Wheat; Drought; osmotic adjustment; heritability.*

1. INTRODUCTION

Wheat (Triticum aestivum L.) is a staple food for more than 35% of the world population and it is also the first grain crop in Egypt. Drought is the most important limiting factor for crop production and it is becoming an increasingly severe problem in many regions of the world. In addition to the complexity of drought itself (Passioura, 1996, 2007).

The objective in many breeding programs is to develop cultivars tolerant to drought stress but success has been limited. Genetic improvement of stress tolerance in crop plants requires identification of relevant physiological stress tolerance mechanisms as selection criteria (Mayer and Gozlan, 1982) and testing to verify the value of such criteria for improvement of stress tolerance. Osmotic adjustment (OA) is generally considered an important component of drought resistance (e.g. Ludlow and Muchow, 1990). Osmotic adjustment (OA) strongly depends on the rate of plant water stress. OA requires time, and fast reduction in plant water status does not allow time for adjustment. This is very significant when genotypes are compared for their OA capacity. However, the importance of the time and the rate of stress for the development of OA imply that OA may not be a very effective mechanism of drought resistance under conditions where the development of drought is by nature very rapid, such on very light tropical or sandy soils of very low water holding capacity (Blum, 1996).

Leaf relative water content (RWC) was abetter indicator of water status than was water potential (Sinclair and Ludlow, 1985). Specific leaf area (SLA), an indictor of leaf thickness, has often been observed to be reduced under drought conditions (Marcelis et al., 1998). In the present work the mechanisms involved in the response to drought were investigated in 22 wheat Lines with contrasting

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drought tolerance capacities, in order to study the relationship between the physiological basis of drought response and plant stress tolerance.

2. MATERIAL AND METHODS

The present investigation was carried out in the greenhouse during 2008/2009 and 2009/2010 at the Institute of Biological Production Systems, Leibniz Universität Hannover, Germany. The breeding materials used in this study consisted of all F5 families selected in F4 on the basis of high yield under drought conditions, as well as parents and the tolerant cultivar (Sahel 1). The total number of evaluated families was 22 families. The used genetic material:

Table 1

The pedigree and origin of the two parents and the tolerant cultivar (Sahel 1).

Parental name	Pedigree	Origin
Sids 4 (P1)	May'S'/Mon'S'//CMH74 A.592/3/Giza 157*2	Egypt
Tokwie (P2)	------	South Africa
Sahel 1	NS 732/PIMA//Veery'S'	ICARDA

Laboratory procedures:

Two experiments were carried out during 2007/2008 and 2008/2009 seasons. Polyvinyl chloride columns of 12.5 cm inner diameter were used and the length of the columns was 50 cm in the two experiments. The substrate for growing the plants was homogenized loamy soil, which was dried in the greenhouse for one month before starting the experiments. While filling the columns, soil samples were taken to determine the initial soil water content. Soil water holding capacity (SWHC) was measured by subtracting the weight of columns filled with dry soil and the weight of columns saturated with water after allowing the exceeding water to drain until there was no change in the weight.

The experiment was designed as RCBD with four replicates in north-south direction, and genotypes were completely randomized within the plots. Two seeds were sown in the middle of every column. After germination, one seedling was removed. Plants were kept well watered at 80% water holding capacity (WHC), Columns were weighed and plants were topped up every third day to reach required SWHC and the drought stress cycle was started four weeks after sowing and the duration of the stress cycle was about 4 weeks. Fertilizer was applied to the optimum dosage and diseases and pests were controlled using appropriate pesticides.

The following measurements were recorded: -

– Physiological traits.

1 - Relative leaf water content (%) (RWC): was measured at the beginning of the stress cycle and at the end of stress cycle on leaf cuttings, a small part of leaf area of the main tiller and the first biggest other tiller were cut and RWC was calculated using the following equation:

$$RWC = (FW - DW)/(TW - DW) \times 100$$

where FW and DW are fresh weight and dry weight of the leaf and TW is the turgor weight of the leaf

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after submergence of leaf samples in distilled water for 24 h.

2 - Osmotic potential (Ψs): was measured at the beginning of the stress cycle and after the stress cycle on leaf cuttings, a small part of leaf area of the main tiller and the first biggest other tiller were cut and stored to measure osmotic potential by using the psych- rometric method and Wescor C-52 sample chambers (Wescor Inc., Logan, USA). Ψs,cd was corrected for relative leaf water content. Osmotic adjustment (Mpa), OA was estimated as follows:

$$OA = \Psi s,cd \times RWCcd - \Psi s,ww \times RWCww$$

where Ψs,cd and RWCcd are Ψs and RWC under drought stress conditions, and Ψs,ww and RWCww are Ψs and RWC under well watered conditions.

3 - Specific leaf area (cm²g-1): was measured after the stress cycle for all leaves and it will be calculated by:

The ratio of leaf area to leaf dry mass (cm²g-1).

Statistical procedures:

1- Analysis of variance for randomized complete block design was carried out according to Snedecor and Cochran (1980).

2 – The genotypic variance $\sigma 2\ g = M2 - M1 / r$

3 – The phenotypic variance $\sigma 2\ p = \sigma 2\ g + \sigma 2\ e$

4 –The genotypic (G.C.V%) and phenotypic (P.C.V%) coefficients of variability were calculated as $\sigma g / \bar{x}$ and $\sigma p / \bar{x}$, respectively .

5 – Heritability in the broad sense (H) was estimated as the ratio of genotypic (σ2g) to the phenotypic (σ2g + σ2e) variance according to Walker (1960).

Table 2

The analysis of variance and expected means of squares

Source of variance	D.F	M. S	E. M. S
Replication	r – 1	M_3	$\sigma^2 e + g\ \sigma^2 r$
Genotypes	g – 1	M_2	$\sigma^2 e + r\ \sigma^2 g$
Error	(r –1) (g –1)	M_1	$\sigma^2 e$

6 - Mean comparisons were calculated by using revised L.S.D where, L.S.D = least significant difference, and was calculated as:

$R\ L\ S\ D\alpha = (t\text{-})\alpha * \sqrt{(2MSE / r)}$ (El Rawi and Khalafalla 1980)

Where t- is the t value from "minimum-average-risk t-table" at F-value of treatments, treatment df and experimental error df.

3. RESULTS AND DISCUSSION

The analysis of variance (Table 3) revealed highly significant differences among the families selected of population I on independent culling levels basis in all physiological traits, i.e., relative leaf water content under well-watered (RWCww) and relative water content under drought stress conditions (RWCcd), specific leaf area under drought stress (SLAcd) and osmotic adjustment (OA) in the two years.

Table 3

Analysis of variance

Years	S.O.V	d.f	Mean Squares			
			RWC_{ww}	RWC_{cd}	SLA_{cd}	OA
2007	Reps	3	18.21	8.95	85.14	0.01
	Families	24	13.46**	13.96**	1892.79**	0.04**
	Error	72	2.99	2.83	36.46	0.006
2008	Reps	3	3.85	1.17	70.38	0.003
	Families	24	8.82**	13.73**	1116.70**	0.05**
	Error	72	3.31	2.89	52.48	0.009

The combined analysis over two years (Table 4) revealed highly significant differences among families and years for the above mentioned traits in the two populations. While, families x years interaction were highly significant for SLAcd and OA and non significant for RWCww and RWCcd in the two populations.

Table 4

The combined analysis of variance over the two years

S.O.V	d.f	Mean Squares			
		RWCww	RWCcd	SLAcd	OA
Year	1	280.54**	116.60**	17196.6**	0.32**
Rep/Year	6	6.32	3.96	67.00	0.01
Families	24	21.36**	24.45**	2765.88**	0.06**
F x Y	24	0.93	3.24	243.60**	0.04**

The range and the mean values of the four studied traits within and across the two years are presented in Table (5). The results showed that average of RWCww was 88.23% with a range from 84.35 to 91.99% in the first season and in the second season, the average was 85.82% with a range from 82.56 to 89.10%. However, mean across years of RWCww was 87.03% with a range from 83.46 to 90.54%. Moreover, the results showed that six families, i.e., no. 6, 22, 33, 38, 43 and 45 and two families, i.e., no. 33 and 38 significantly exceeded the best parent in the first and the second seasons, respectively. While, ten families, i.e., no. 6, 13, 22, 23, 25, 33, 36, 38, 43 and 45 and six families, i.e., no. 6, 22,

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33, 36, 38 and 45 were significantly higher than the check in the first and the second seasons, respectively.

Table 5

The range and the mean values for the physiological traits within and across years.

Trait	Year	Range	Means±S.E
RWCww (%)	2007	84.35 – 91.99	88.23±0.25
	2008	82.56 – 89.10	85.82±0.22
	Mean	83.46 – 90.54	87.03±0.18
RWCcd (%)	2007	80.41 – 87.30	84.04±0.24
	2008	79.17 – 86.35	82.51±0.23
	Mean	80.33 – 86.83	83.28±0.18
SLAcd (m2kg-1)	2007	81.33 – 165.32	125.77±2.21
	2008	85.15 – 144.58	107.36±1.76
	Mean	86.51 – 154.95	116.56±1.56
OA (Mpa)	2007	-0.36 – -0.79	-0.56±0.01
	2008	-0.23 – -0.68	-0.47±0.01
	Mean	-0.37 – -0.68	-0.51±0.01

The average RWCcd (Table 5) was 84.04% with a range from 80.41 to 87.30% and from 79.17 to 86.35% with an average 82.51% in the first and second season, respectively. Mean over all for RWCcd was 83.28% with a range from 80.33 to 86.83%. Three families (no. 22, 33 and 36) and two families (no. 22 and 33) were significantly higher than the best parent in the first and the second seasons, respectively. Meanwhile, three families (no. 22, 33 and 36) and four families (no. 22, 25, 33 and 36) surpassed the check (Sahel 1) in the first and the second seasons, respectively (Table 6). These results are in agreement with those obtained by, Tahara et al. (1990), Morgan (1995), Siddique et al. (2000) and Suprunova et al. (2004). González et al. (2008) cleared that the range of leaf relative water content varied from 50.03 for ND66 to 72.02% for L31under terminal water-stress condition.

Mean SLAcd (Table 5) was 125.77 m2kg-1with a range from 81.33 to 165.32 m2kg-1 and from 85.15 to 144.58 m2kg-1 with an average 107.36 in the first and second seasons, respectively. However, mean across years of SLAcd was 116.56 m2kg-1 with a range from 86.51 to 154.95 m2kg-1. Twelve families, i.e., no. 6, 19, 22, 23, 24, 25, 26, 28, 33, 38, 45 and 46 in the two years were significantly higher than the best parent. While, twelve families, i.e., no. 6, 19, 22, 23, 24, 25, 26, 28, 33, 38, 45 and 46 in the first season and all selected families except (no. 13, 37, 39 and 43) in the second season surpassed the check (Table 7).

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Table 6

The average of relative leaf water content under normal (RWCww) and drought (RWCcd) conditions across the two years.

Selected families	RWC_{ww}			RWC_{cd}		
	Year 1	Year 2	Mean over all	Year 1	Year 2	Mean over all
1	87.50	85.36	86.43	85.06	83.57	84.31
6	90.43	87.20	88.81	82.51	81.19	81.85
13	89.35	86.19	87.77	85.90	82.48	84.19
19	87.99	84.55	86.27	84.90	84.19	84.54
22	89.85	87.20	88.52	86.69	85.37	86.03
23	88.83	86.81	87.82	83.18	82.12	82.65
24	86.08	84.79	85.44	80.41	82.21	81.31
25	88.83	86.21	87.52	85.69	84.59	85.14
26	85.54	83.24	84.39	82.68	81.34	82.01
28	86.44	85.34	85.89	81.48	79.17	80.33
33	91.99	89.10	90.54	87.30	86.35	86.83
36	89.44	87.30	88.37	87.09	84.74	85.92
37	87.59	86.03	86.81	84.55	83.77	84.16
38	90.43	87.55	88.99	84.91	82.42	83.66
39	88.38	86.31	87.34	84.84	83.33	84.09
42	87.28	85.00	86.14	83.11	82.49	82.80
43	89.68	86.44	88.06	85.96	81.56	83.76
45	90.94	87.18	89.06	83.99	81.54	82.76
46	87.45	85.74	86.59	82.79	83.60	83.20
48	86.07	83.33	84.70	81.35	79.87	80.61
55	86.64	84.66	85.65	82.49	79.43	80.96
62	84.35	82.56	83.46	82.02	79.96	80.99
Average	88.23	85.82	87.03	84.04	82.51	83.28
P1	87.10	84.47	86.04	83.02	82.47	82.74
P2	86.34	83.95	85.15	83.65	81.14	82.40
Sahel 1	86.24	84.02	85.38	83.62	82.12	83.37
$RLSD_{0.05}$	2.51	3.02	--	2.44	2.46	--
$RLSD_{0.01}$	3.31	4.14	--	3.22	3.26	--

The average of osmotic adjustment (OA) was –0.56 Mpa with a range from –0.36 to –0.79 Mpa and from –0.23 to –0.68 Mpa with an average –0.47 Mpa in the first and second seasons, respectively. Moreover, mean over all was –0.51 Mpa with a range from –0.37 to –0.68 Mpa (Table 5). Moreover, five families (no. 22, 25, 26, 33 and 48) and three families (no. 22, 33 and 38) significantly exceeded the best parent in the first and the second seasons, respectively. While, five families, i.e., no. 22, 25, 26, 33 and 48 and four families, i.e., no. 19, 22, 33 and 38 surpassed the best parent in the first and the second seasons, respectively (Table 7). The wide range of variability among genotypes also indicates the suitability of OA as selection tool for breeders under water deficit environments. These results

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were in agreement with these obtained by Morgan (1977b, 1995, 1999), Morgan et al. (1986), Blum et al. (1999) and Moinuddin et al. (2005). Also, Martin et al. (2009) Also, Ref. [10] showed that Tullio, the drought susceptible Italian genotype, had an osmotic adjustment of –0.63 MPa, four times greater than in Pandas, though this drought resistant cultivar showed a similar content in osmotically active substances.

Table7

The average of specific leaf area under drought conditions (SLAcd) and osmotic adjustment (OA) across the two years.

Selected families	SLAcd			OA		
	Year 1	Year 2	Mean over all	Year 1	Year 2	Mean over all
1	153.83	121.38	137.60	-0.40	-0.33	-0.37
6	99.03	85.15	92.09	-0.59	-0.40	-0.50
13	148.85	127.16	138.00	-0.55	-0.51	-0.53
19	127.48	112.22	119.85	-0.36	-0.59	-0.48
22	116.72	97.46	107.09	-0.67	-0.68	-0.68
23	119.25	102.41	110.83	-0.53	-0.31	-0.42
24	81.33	91.69	86.51	-0.61	-0.54	-0.58
25	101.81	87.51	94.66	-0.79	-0.49	-0.64
26	123.78	117.98	120.88	-0.65	-0.57	-0.61
28	88.77	90.89	89.83	-0.51	-0.31	-0.41
33	106.52	85.93	96.22	-0.72	-0.63	-0.67
36	135.43	100.62	118.02	-0.51	-0.45	-0.48
37	142.71	131.76	137.24	-0.58	-0.50	-0.54
38	115.66	99.70	107.68	-0.57	-0.61	-0.59
39	165.32	144.58	154.95	-0.54	-0.23	-0.38
42	146.30	118.55	132.43	-0.59	-0.44	-0.51
43	148.89	135.83	142.36	-0.38	-0.53	-0.45
45	106.34	88.08	97.21	-0.52	-0.50	-0.51
46	118.95	97.28	108.11	-0.43	-0.46	-0.45
48	133.08	96.13	114.60	-0.68	-0.39	-0.53
55	132.11	108.96	120.53	-0.56	-0.33	-0.45
62	154.68	120.70	137.69	-0.51	-0.54	-0.52
Average	125.77	107.36	116.56	-0.56	-0.47	-0.51
P1	149.60	119.70	134.70	-0.44	-0.48	-0.46
P2	126.00	108.70	117.30	-0.53	-0.43	-0.48
Sahel 1	126.00	114.50	120.30	-0.54	-0.46	-0.50
$RLSD_{0.05}$	4.47	9.48	--	0.11	0.13	--
$RLSD_{0.01}$	9.78	12.47	--	0.14	0.17	--

The phenotypic (P.C.V.%) and genotypic (G.C.V.%) coefficients of variation and heritability (h2) estimates for all studied traits in the first (2007) and the second (2008) seasons are presented in Table (8). The results showed that the phenotypic and genotypic coefficient of variation were (2.68 and 1.83 %) and (2.52 and 1.37 %) for RWCww, (2.82 and 1.98 %) and (2.87 and 2.00 %) for RWCcd, (17.79 and 17.13 %) and (16.62 and 15.19 %) for SLAcd and (25.26 and 19.62 %) and (27.20 and

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19.85 %) for OA in the first and second seasons, respectively.

Table 8

Phenotypic coefficient of variation (PCV%), genotypic coefficient of variation (GCV%) and broad sense heritabilities (h2) for all studied traits.

Traits	Year	PCV%	GCV%	h^2
RWC_{ww}	2007	2.68	1.83	46.68
	2008	2.52	1.37	29.39
RWC_{cd}	2007	2.82	1.98	49.58
	2008	2.87	2.00	48.39
SLA_{cd}	2007	17.79	17.13	92.72
	2008	16.62	15.19	83.52
OA	2007	25.62	19.62	58.62
	2008	27.20	19.85	53.25

The broad sense heritabilities (Table 8) were (46.68 and 29.39%) for RWCww, (49.58 and 48.39 %) for RWCcd, (92.72 and 83.52 %) for SLAcd and (58.62 and 53.25 %) for OA in the first and second seasons, respectively. The high heritability estimates obtained for both SLAcd and OA provide evidence for the effectiveness of selection for both characters in improving drought tolerance. These results are in accordance with those obtained by Tahara et al. (1990), Dhanda and Sethi (1996), Slafer and Araus (1998), Golparvar (2003) and Golparvar et al. (2006).

Our results were derived from test conditions that ascribe an advantage to OA capacity. They lend full support to results presented by Morgan, (1977, 1995, 1999) showing that wheat lines can differ consistently for OA. It is therefore concluded that OA can be an important component of drought resistance in wheat within a relevant environmental context.

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EFFECT OF SEED GENERATION AND TYPE OF ORGANIC MANURE SOLUTION ON GROWTH AND YIELD OF HYBRID EGGPLANT

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Abstract

The experiment was conducted during the growing season of 2008-2009 at Kufa district in Najaf Governorate to investigate the response of Barcelona eggplant hybrid to seed generation (F1, F2) and 6 treatments of organic manure by mixing with water 1:10 v/v (i.e. poultry, Cow , Sheep, and broad bean manure). The above organic solutions were applied with rate of 3L/m² for 4 times in 60 days interval, while Chinese Humi Max manure was mixed with water by 1:200 v/v and applied with rate of 100 cm³/m² at the same intervals. The F1-seed generation gave the highest growth rates and fruit yield as compared with F2-generation. Also, the obtained results showed that the application of poultry and sheep manure solution increased significantly the growth and fruit yield as compared with other treatments. The favorable effects on growth and fruit parameters were obtained when growing eggplant by F1-generation and application of poultry or sheep solution.

Key words: *seed generation; manure solution; olanum melongena; eggplant; growth; yield*

INTRODUCTION

Eggplant (Solanum melongena L.) is a member of solaneceae family . It is one of the most important and popular oriental vegetable crops grow in Iraq for local consumption. The fruits are excellent source of roughage, carbohydrates, protein, vitamins A, B, C, potassium, iron and besides that medical benefits for patients of diabetes , asthma , gout and cholesterol (Matloub et. al.1989; Daunay et.al.2000).

Plant Production depends on many factors, the most important factor is variety and plant nutrition with compositing organic manure which is not available in high quantities in most countries. Therefore, the organic manure should be provided through liquid solution in order to economized and utilized in the proper mean.

The choosing of eggplant hybrids based on some factors, which enable to ensure high yield fruit quality besides the endurance of environmental variation and tolerance of pesticides. Generally F1-hybrids distinguished by hybrid vigor phenomenon, the studies are demonstrated that the genetic segregation does not happen in F1-individual plants, if the using parents are pure, while the self-pollination between first generation caused to produce second generation.

It is worth mentioning; that hybrid seed prices mostly are high due to the cost of pure line and the technical process of production. So, many companies are working to produce F2-seed generation (Riggs 88; Ingale and Patil 1997; Daunay 2007). Numerous studies indicate that organic manure provides the plant with most nutrient elements and the element deficiency rarely happened when applied. In addition it will improve the physical, chemical, and biological properties of soil, which will be inversed the availability of soil nutrient elements especially with alkaline reaction such as Iraqi soils (Tisdale et.al.1993; Seen and Kingman 1998; Eghball 2002). Increasing plant growth and yield due to liquid manure application have been reported by many workers on different crops (Kadhum

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et.al. 1987; Al-Moshileh and Motawei 2007) on eggplant, (Kadhum et. al. 1989; Burgrs and Levins 2007) on pepper, (Bohme and Thilua 1997) on tomato. Humic and folvic acids application might successfully be used in low concentration to improve soil characteristics, plant nutrition. The growth and yield of pepper and tomato had been increased by fertilization of humic acid (Ertam Yildirim 2007;Yasar. Karakurt 2009).

The main objective of this study is to evaluate the performance of two seed generation (F1, F2) of Barcelona eggplant hybrid and the effect of 6 treatments of composting manure solution on the growth and fruit yield.

MATERIALS AND METHODS

The study was conducted in 2008-2009 at private field Kufa district of Najaf Governorate, air composting method had been used to prepare the poultry, cow, sheep, and broad bean manure which should be dried and ground. The manure were compacted on layer in trough with dimension (2 * 2 * 1.5 m**),** urea fertilizer was added by rate 1% of manure weight to activate the decaying processes. The heap should turnover two times per week to prevent temperature increasing. The decaying of animal manure had done after 3 months, while the broad bean manure decayed after 6 months. The color of manure became brown without ammonia odor (Hayes and Clap 2001). The table (1) shows some chemical characters of different organic manure before and after composting according to (A.O.A.C. 1975).

Table (1) some chemical properties of different organic manure before and after composting.

Organic manure	Chemical characters						
	Ec.of 5:1 $dS.m^{-1}$	pH	Carbon $g.kg^{-1}$	Nitrogen $g.kg^{-1}$	C/N ratio	P2O5 $g.kg^{-1}$	K2O $g.kg^{-1}$
	before composting						
Poultry	9.27	8.14	275	29.5	11.5	12.2	9.3
Cow	7.24	7.36	317	19.4	16.7	7.5	15.3
Sheep	10.67	7.12	345	16.7	21.5	9.3	18.6

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Broad bean	8.50	9.32	355	11.8	36.5	6.5	10.1
	After composting						
Poultry	1.65	6.28	255	30.9	9.5	14.2	23.4
Cow	1.71	6.53	300	28.0	10.7	8.7	21.4
Sheep	1.45	6.80	330	27.3	12.2	10.3	27.1
Broad bean	2.26	7.22	350	16.5	21.4	7.9	17.5

The experiment was arranged in split plot design with three replicates, where eggplant seed germination (F1 , F2) were arranged randomly within the main 2 plots, while organic manure treatments distributed in 6 sub-plots as control, cow, sheep, poultry , Humi Max-china origin and broad bean, which represented with symbol (S0, S1, S2, S3, S4, and S5) respectively. The F1-seed generation was imported from semillas company-Spain, while F2- generation produced locally in Kufa district.

The different manure solution was prepared by mixing with water at ratio (1:10) v/v (IFOAM 2000). The solution was applied with rate of (3 L/m²) for 4 times in 60 days interval by adding the solution in the bottom of ridge. The first discharge was added in 50% of plant flowering stage. The Humic Max liquid solution was mixed with water by (1:200 v/v) and applied with rate of (100 cm³/m²) according to the above method.

Eggplant hybrid seeds of Barcelona were sown on 25 July in 209 cell foam trays filled with mixture consisted of 1:2 of sandy soil and peat moss, seedling were planted 40 cm apart on both sides of flat board 160 cm width. Each experimental plot included 40 plants with net area of (12.4 m²). All agricultural practices were carried out accordingly.

Five plant were chosen randomly from each sub plot at 160 day after transplanting to record the growth rate , while the fruit yield obtained by harvesting from 24.11.2008 until 3.6.2009. The first 5 harvest considered as early yield. The obtained data were statistically analyzed and means separation were done using Duncan multiple test at 0.05 level according to SAS program (SAS 2000).

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RESULTS AND DISCUSION

Vegetative growth characters

The growth rates of plants were significantly greater at F1- generation than F2 (table 2). The increasing percentage for leaves number, branches number, leaves area, dry weight of leaves and stem and total chlorophyll content were 10.89, 4.59, 57.49, 29.52, 22.88, and 9.55% respectively at F1-generation than F2. This is due to the homogeneity of F1-plants in growth according to genetic constitution and existence of hybrid vigor phenomenon. The obtained results are in agreement with those of (Madal and Dama 1993), where reported that F1 growth of eggplant was significantly greater than F2 of the same hybrid.

The results also show that the growth rates of the plants increased significantly with application of organic manure solution. The highest value of growth were obtained at poultry solution (S3) followed by sheep solution (S2), while the lowest values were recorded by the control plants (S0). These results may be due to the role of organic manure in improving the chemical, physical, and biological characters of soil (Tisdale et. al. 1993). In addition to decaying processes of different manure before they are applied may be contributed to availability of nutrient element such as NPK (table1), that are needed for optimal plant growth and development (Eghball 2002).

Regarding to the interaction effect, there were significant differences in all growth parameters, it could be stated that the highest growth rates were recorded by (F1S3) followed with (F1S2) treatment.

Table (2) Effect of seed generation, different manure solution and their interaction on vegetative character of eggplant.

characters	Leaf No.	Branch No.	Leaf area	Dry weight		Total chlorophyll
				Leaves	Stems	
Treatments	Effect of seed generation					
F1	65.21 a	6.11 a	4505.04 a	76.92 a	86.43 a	17.29 a
F2	58.81 b	5.48 b	2860.38 b	61.71 b	70.33 b	15.78 b
	Effect of manure solution					
S0	50.65 e	4.41 e	2363.88 e	56.18 e	60.42 f	12.10 d

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S1		64.26 c	6.08 c	3790.28 c	70.55 c	78.07 d	17.24 b
S2		66.78 b	6.71 ab	4502.75 b	74.83 b	84.89 b	17.87 a
S3		68.91 a	6.97 a	5105.51 a	82.49 a	92.12 a	18.86 a
S4		65.35 c	6.51 b	3547.30 c	71.72 c	82.73 c	17.68 a
S5		56.11 d	5.19 d	2786.65 d	60.13 d	72.06 e	15.96 c
		Effect of the interaction					
F1	S0	55.20 f	4.49 e	2960.80 f	59.53 g	67.46 h	12.58 f
	S1	66.66 b	6.07 c	4755.56 c	78.29 c	86.88 c	18.25 b
	S2	69.56 a	6.95 ab	5357.03 b	84.96 b	92.58 b	18.51 b
	S3	71.60 a	7.26 a	6060.13 a	94.33 a	101.62 a	19.79 a
	S4	67.93 b	6.76 b	4416.46 c	79.27 c	91.44 b	18.27 b
	S5	60.33 e	5.31 d	3480.26 e	65.17 e	78.60 e	16.32 c
F2	S0	46.10 g	4.33 e	1766.96 i	52.83 i	53.38 j	11.63 g
	S1	61.86 d	6.08 c	2825.00 f	62.81 f	69.27 g	16.24 d
	S2	64.00 c	6.48 bc	3648.46 e	64.69 e	77.20 e	17.22 c
	S3	66.23 b	6.69 b	4150.90 d	70.65 d	82.62 d	17.46 c
	S4	62.76 c	6.26 bc	2677.93 g	64.18 e	74.01 f	17.10 c
	S5	51.90 f	5.07 d	2093.03 h	55.09 h	65.52 i	15.60 e

Fruit yield and quality

Data presented in table (3) demonstrated that there were significant differences in yield and fruit quality between the plant of F1 and F2. The increasing percentage of fruit dry matter, fruit number/plant, fruit weight, early yield, and total yield were 10.81, 8.54, 18.59, 22.53, and 28.99% respectively at F1 generation than F2. The obtained results might be due to genetic constitution of F1-generation and existence of hybrid vigor phenomenon expressed with homogeneity of plant growth. These results were in accordance with those mentioned by (Daskalov et. al. 1974) concerning the eggplant of F1-generation.

All treatments of organic manure solution had a significant effect on fruit yield parameters except S4 and S5 treatments regarding the early yield. The highest rates of these characters was recorded by poultry solution (S3) followed with sheep (S2). These results might be due to the distinguish percent of NPK in poultry and sheep manure as compared with other manure , in addition to humic acids, organic matter and hormones, that have important role on growth physiological processes. These

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effects were expressed on yield and fruit quality. The obtained results were in agreement with (Kadhum et. al. 1989; Al-Moshileh and Motawei 2007; Burgis and Levins 2007).

Table (3) Effect of seed generation, different manure solution and their interaction on yield and fruit quality of eggplant.

characters		Dry matter%	Fruit No./plant	Fruit weight(g)	Early yield kg/m²	Total yield kg/m²
Treatments		Effect of seed generation				
F1		10.81 a	33.28 a	174.99 a	4.73 a	18.24 a
F2		9.18 b	30.66 b	147.55 b	3.86 b	14.14 b
		Effect of manure solution				
S0		8.18 d	29.81 e	151.46 e	4.26 b	14.11 e
S1		9.99 cd	30.94 d	160.52 c	4.17 c	15.55 c
S2		10.60 b	33.09 b	167.19 b	4.51 a	17.30 b
S3		12.06 a	35.86 a	173.86 a	4.57 a	19.61 a
S4		10.08 c	31.65 c	160.33 c	4.28 b	15.86 c
S5		9.04 d	30.48 d	154.25 d	3.98 d	14.71 d
		Effect of the interaction				
F1	S0	8.64 g	30.73 d	163.04 f	4.14 f	15.63 f
	S1	10.75 c	32.25 c	172.97 d	4.66 d	17.40 c
	S2	11.56 b	33.99 b	181.30 b	5.00 b	19.23 b
	S3	13.59 a	38.73 a	191.90 a	5.40 a	23.18 a
	S4	10.83 c	32.29 c	174.36 c	4.81 c	17.56 c
	S5	9.47 f	31.70 d	166.38 e	4.37 e	16.45 d
F2	S0	7.71 h	28.89 f	139.88 k	4.37 e	12.60 k
	S1	9.23 f	29.64 e	149.07 i	3.69 gh	13.69 i

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	S2	9.64 c	32.18 c	153.09 h	4.01 f	15.37 g
	S3	10.54 d	33.00 bc	155.82 g	3.75 g	16.04 e
	S4	9.33 f	31.01 d	146.30 j	3.75 g	14.15 h
	S5	8.62 g	29.26 e	142.13 j	3.58 h	12.97 j

Significant interaction effect was observed on all fruit parameters, and it could be mentioned that the plant of F1 generation and application of poultry manure recovered the highest values of fruit dry matter, fruit number/plant, fruit weight, early and total yield, where the values was 13.59%, 38.73 fruit, 191.90, 5.40 kg/m², and 23.18 kg/m² respectively followed with F1S2.

CONCULUSION

It could be concluded that F2 seed generation can be successfully used in case of the unavailability to provide with F1-generation, besides the application of poultry or sheep manure solution to obtain the highest vegetative growth parameter and fruit yield.

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TRANSMISSION OF BEAN YELLOW MOSAIC VIRUS IN RELATION TO APHID SPECIES (HOMOPTERA: APHIDIDAE)

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Abstract

Experiments were carried out to determine the periods of acquisition, latent period, inoculation and retention of bean yellow mosaic virus (BYMV) when transmitted by the green peach aphid. Trials were set up with broad bean plants, and the method of indirect-ELISA was used to determine the latent carriers of the virus. Even a single viruliferous aphid was capable of transmitting the virus. However, the maximum transmission was obtained when 10 aphids per plant were employed. It was established that after acquisition of BYMV, Myzus persicae remains short time. The optimal period for acquisition of BYMV by the green peach aphid is determined to be 48 hours, and the minimal is 45 minutes. The optimal latent period of the virus in the vector for inoculation of BYMV by the green peach aphid is 48 hours and the minimal is 20 minutes.

Key words: Bean yellow mosaic virus (BYMV)- *Myzus persicae*

INTRODUCTION

Faba bean (*Vicia faba* L.) is among the most important edible crops that supply Egyption people with protein, consumed as dry seeds, green vegetable and as processed food. Moreover, it is essential for feeding the live stock on dry seeds, as well as green and dry straw **(Hassan, 1996)**. Assiut governorate is considered as one of the famous area cultivating with faba been in Egypt. Insect pests, specially aphids, cause severe crop losses to faba bean, principally by direct feeding damage or as a vector and disseminator to virus disease (Manaa *et al*, 1999). Virus diseases, considered as a serious worldwide problem **(Jones, 1997)**. The infection with certain viruses causes significant economic losses and yield reduction (estimated by 30% on susceptible cultivars of faba bean cultivars) **(Khalil & Erskine, 2001)**. Bean yellow mosaic virus (BYMV) consider as one of the most devastating viruses affecting faba bean plants in Egypt **(El-Beshehy, 1999 and Radwan *et al*., 2007)**. BYMV (family Potyviridae, genus Potyvirus) is an aphid transmitted virus in non-persistent mode and has a wide host range **(Jones and Smith, 2004 & 2005)**. It causes a considerable destructive in seed yield, particularly, when plants were infected at the beginning stage of faba bean at the growing regions of Egypt. Systemic symptoms caused by BYMV infection do not kill faba bean plants, but have the potential to spread faster and further into the crop, causing greater overall yield reduction, despite inducing milder symptoms **(Sidaros *et al*., 2006)**. BYMV are transmissible by mechanical inoculation and by aphid vectors in a non-persistent manner. More than 40 aphid species can transmit BYMV, including *Acyrthosiphon pisum, Aphis craccivora, Aphis fabae, Macroszphum euphorbiae* and *Myzus persicae* **(Bos, 1970)**.

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The main propose of this work is to examine the ability of some aphid species to transmit and disseminate BYMV on faba bean, as well as certain relationship among them.

MATERIALS AND METHODS

The studies were conducted during the 2011 / 2012 and 2012/ 2013 seasons and were located at the green house of Faculties of Science, El-Azhar and Assiut University.

1-PLANTS

All the tested plants used to maintain the virus isolates and virus free aphid were grown in an artificial soil mix. It consisted of 1:1, peat moss: sand. Normal Hoaglands solution (Hewitt 1966) was watered into the mix once or twice weekly as necessary. The plants were grown in insect screened glasshouses and were treated weekly with insecticide. Giza cv461 faba bean was used. *Chenopodium quinoa* plants were used as indicator host for test inoculum of the BYMV. Seeds were sown in a mixture of sand and clay (1: 2 v/ v) in plastic pots (10 cm in diameter).

2-VIRAL ISOLATION

Twenty four samples of faba bean plants exhibiting virus-like symptoms were collected from eight different fields at Assiut Governorates. These samples were checked serologically against BYMV, alfalfa mosaic virus (AMV) and broad bean mosaic virus (BBMV) according to Hobbs *et al.,* 1987. These antibodies were obtained from the Agricultural Genetic Engineering Research Institute (AGERI), Egypt.

Samples giving only BYMV positive results were mechanically inoculated on *C. quinoa* and then isolated by single local lesion according to El-Afify *et al*., 2007. The virus isolate was maintained on broad bean plants under greenhouse conditions for subsequent experiments.

3- REARING INSECTS:

Two sets of healthy Faba bean plants were sorted in two cages (36 x 40 x 60 cm), to maintain the virus- free aphid colonies. Two species of *Aphid craccivora* and *Myzus persicae* were collected from broad bean plants to obtain stocks of virus- free aphids. The plants were renewed weekly in each cage.

4-INSECT TRANSMISSION:

Two aphid species were used to compare the vector specificities to BYMV isolate. Insect from each aphid culture were starved for 24 hours at 4ºC. Subsequently, the aphids given acquisition access three to five minutes to *V. faba* leaves infected with the virus isolate in Petri dishes and then transferred to healthy broad bean plants for 24 hours. They were then sprayed with an insecticide before their return to the glasshouse. The experiments were repeated four times. The aphid-isolate combination involved ten aphids per plant and ten plants per treatment. Probability of transfer (p) was calculated using the function of Burrows (1987):

$$p = 1-[(2kR + k-1)/(2kN + k-1)]^{1/k}$$

where: N = number of test plants, R = number of healthy plants, and k = number of aphids transferred to a single host plant.

COMPARISON OF APHID SPECIES

Aphids from each colony were tested for their ability to transmit BYMV. *V. faba* plants were used as virus sources and as test plants. Each test was done in duplicate using ten aphids per plant. Acquisition and inoculation access periods were three days of each. Effect of number of each aphid on virus

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transmission were allowed to feed on infected plants for 72 hours and then placed singly or in groups of five or ten on healthy broad bean seedlings. Subsequently, the aphids were killed with insecticide.

PERSISTENCE OF BYMV ON APHIDS:

Virus-free colony of *A. craccivora* and *M. persicae* given acquisition access to infected *V. faba* plants for 72 hours. Twenty aphids were then transferred singly onto new sets of healthy test plants every 24 hours until all the aphids had died. The stage of development of each aphid was recorded daily after they were transferred.

ACQUISITION THRESHOLD PERIOD:

A free- virus individuals of *A. craccivora* were given acquisition access for 10, 20, 30, 45 minutes or 1, 2, 4, 6, 12, 24, 48 or 72 hours. At the end of the respective acquisition access periods, the aphids were placed in groups of 10 aphids per plant of the healthy test plants until six days after commencement of the experiment.

INOCULATION THRESHOLD PERIOD:

Non-viruliferous individuals of *A. craccivora* were fed for 72 hours on infected plants and then transferred in groups of 10 to each test plant (ten test plants were used). The aphids were given access to the test plants for either 10, 15, 20, 25, 30 or 45 minutes or 1, 2, 4, 6, 12, 24, 48 or 72 hours. The aphids were killed as mentioned above.

RESULTS AND DISCUSSION

VIRUS ISOLATION

One collected samples which gave positive reaction against BYMV antiserum was only used as virus source to inoculate the plants of *C. quinoa* as a differential host. Inoculated *C. quinoa* leaves showed chlorotic local lesions. The virus was isolated via single chlorotic local leasion technique on faba bean three times.

Variations of symptoms were appeared after aphid transmission. The terminal bud twisted slightly after 6-7 days of inoculation, followed by initiation of very narrow leaves from new buds and was more evident after 15 days of inoculation (Fig. 1). As the time advanced, proliferation of both terminal and axillaries branches occurred resulting into long and thin branches consisting of very narrow leaves. The leaves below the proliferated branches exhibited yellowing or inter venial chlorosis or mosaic. The overall height of the plant was reduced. Such plants produced very few distorted flowers that converted to very small pods.

APHID TRANSMISSION

Result obtain in table (1) and Fig. (1) show that the individual of the two examined aphid species were able to transmit BYMV isolate. The *M. persicae* was able to transmit it more readily (82%) than the *A. craccivora* (70%). The efficiencies with the two aphids to transmit BYMV via individual insect were often at variance. *M. persicae* was more effective in transmitting BYMV (0.19). The work of Makkouk *et al.* (1990) reported that the virus affecting faba bean in West Asia and North Africa was identified as Broad Bean Wilt Faba virus (BBWV). The isolates from Syria and Egypt found to be positively transmitted non- persistently by 4 aphid species, most efficiency by *Myzus persicae*. The work of Su *et al*. (1989) declared that *M. persicae* was more suited to the transmission of BBWV than that of *A. cracivora*, even both of two species have the ability to disperse and transmit the virus. Manna *et al*. (1999) in their study on BBWV at Assiut locality found that two previous mention species were found on the infested faba bean plants. Therefore, the dominant density of *A. craccivora*

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blamed it as responsible of virus dissemination. *A. cracivora* was the most dominant species during the two successive season of study. Several small colonies of *M. persicae* were scattered within those of *A. cracivora.* Therefore, the dominant density of *A. cracivora* blamed it as responsible of virus transmission.

Figure (1): Mosaic and vein-banding symptoms induced on broad bean leaves after M. persicae inoculation.

The relation among the density of aphid individuals on the host plant and the successful transmission of virus was estimated, data recorded in table 2. The rate of transmission increased when larger numbers of aphids were transferred from virus source to test plants. One hundred percent of transmission was obtained when ten *M. persicae* were allowed to feed on each test plant. These experiments indicated that *M. persicae* was efficient vectors of BYMV from infected broad bean plants to healthy ones, the percentage of transfer by a single aphid averaged 36 as compared with 12 in *A. crassefora*. The experiment showed that a single aphid is able to transmit BYMV to a healthy plant. This result confirms the data of Curpetino (1995) who reports that after a single aphid acquires the potato leaf roll virus (PLRV) from a potato plant, it is able to transmit it to a healthy *Physalis floridana* test plant.

Our results revealed that this virus disease has more chance to disseminate at high abundant level of aphid infestation. Also, the result was agreement with Lampert *et al*., 1988, they reported significant results in tobacco etch virus (TEV) transmission by increasing aphid numbers per plant.

The acquisition access period (AAP) and the inoculation access period species were timed and illustrated in Table 3. The results suggested the suitable timing of these indicators when *M. persicae* was tested as BYMV vector. No transmission of BYMV was recorded when AAP was less than 45 minutes (Table, 3). Increasing the AAP beyond this time increased the efficiency of transmission and the optimal AAP was at least 24-48 hours. Similarly, *M. persicae* was able to transfer BYMV within 20 minutes after becoming infective. The rate of transmission increased as IAP was extended up to 6 hours. The *M. persicae* transmitted the BYMV with 100% efficiency when the IAP was 12 hours. These results revealed that *M. persicae* seems to be a successful vector and disseminator agent for BYMV. Aphid transmission of non-persistent viruses is almost completely restricted to very brief (5-10 seconds) intracellular punctures of epidermal or mesophyll cells (Lõpez *et al*., 1969; Powell, 1991). A model in which egestion through the food canal injects virus into the plant does not take account of the anatomical fact that the food and salivary canals in the maxillary stylets fuse at 2-8 µm from the tips (Forbes, 1969).

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In test to examine the persistence transmission of virus isolate by *M. persicae*, it was found that 5 of 13 aphids acquired virus and infected test plants. The aphids retained their infectivity after transfer to test plants and only one insect did so after developing into adult alatae. In the serial transfer tests, individual aphids varied considerably in their abilities to transmit the virus. Transmission usually occurred intermittently. *M. persicae* was efficient in virus transmission only during the first ten days after the commencement of the acquisition access period and then largely lost its transmitting ability at the subsequent transfer periods.

Table (1): The ability of Aphid species to transmit BYMV by aphid species.

Aphids	% transmission	Transmission efficiency*
Aphis craccivora	14/20 (70)**	0.12
Muzus persicae	19/22 (82)	0.19

*Probability of single aphids for transmission BYMV isolate.

**Number of infected plants/number of treated.

Table (2): Transmission (%) of BYMV by different numbers of aphids.

Aphids species	No. of aphid/plant		
	1	5	10
Aphis craccivora	12	73	92
Muzus persicae	36	84	100

Table (3): Effect of different acquisition and inoculation access periods on the transmission of BYMV by M. persicae.

Period	Transmission (%)	
	Acquisition	Inoculation
10 min	0.0	0.0
20 min	0.0	18.0
30 min	0.0	27.0
45 min	3.0	32.0
1 hr	5.0	48.0
1.5 hr	11.0	53.0
2 hr	12.0	86.0
4 hr	15.0	88.0
6 hr	38.0	95.0
12 hr	75.0	100
24 hr	81.0	100
48 hr	100	100

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LANDSCAPE PROTECTION IN LESS DEVELOPED REGIONS WITH A FOCUS ON WETLANDS

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Abstract

Van province has a rich diversity of natural landscape and wetlands due to the combination of natural features such as inactive volcanic mountains, dynamic topography, rich hydrological system and harsh climate. This paper discusses the importance and the need for the integration of natural landscape dimension on the wetland ecosystems case to the regional and local scale plans on the example of Van Province. In this context, natural landscape components with a focus on wetlands and ongoing wetland management efforts in Van, and current development plans and strategies for the province and the region were analysed. Results indicate that natural landscape in general terms and wetlands in particular are neglected themes in the development plans and strategies for the region, and in the reflections to the local scale plans and practices. It is suggested that an integrated framework for development goals in different sectors, landscape protection and wetland management would be more functional in terms of ecologically sustainable development and quality of life in Van.

Key words: *landscape protection, wetlands, Van (Turkey), regional development plans, developing regions*

1. INTRODUCTION

Wetland landscapes deliver a wide range of critical and important services such as provisioning, regulating, supporting and cultural which are vital for human well-being. Maintaining the natural functioning of wetland landscapes will enable them to continue to deliver these services and to function as one of the key life supporting system of Earth (Durigon et al. 2012; Ramsar Convention Secretariat 2010). The degradation and loss of wetlands is more rapid than that for other ecosystems on the Earth. Losses tend to be more rapid where populations are increasing most, where demands for increased economic development are greatest (Ramsar Convention Secretariat 2010) and where wetlands perceived almost useless. The projected continued loss and degradation of wetlands will result in further reduction in human well-being, especially for poorer people in less developed countries and regions where technological solutions are not as readily available, environmental awareness among public is very low and rapid economic development is the leading priority for public. In this framework, policy intervention failures that are due to the lack of consistency among government policies in economics, environment, natural resource management, physical planning (Turner et al. 2000) are some of the most important reasons of threats to ecological sustainability of wetland landscapes. Lack of consistency among policies is related with the different priorities of the development strategies as well as with the lack of awareness on various benefits of wetland landscapes to society in different levels and contexts. Planners and decision-makers at many levels are frequently not fully aware of and not consider the connections among wetland condition and the provision of wetland services and, landscape planning and spatial planning and, the consequent benefits for people. This lack of understanding and recognition leads to ill-informed, short- termed and narrow-scoped

decisions on regional and local development, which contribute to the rapid loss, conversion and degradation of wetland landscapes and their natural and cultural functions (De Groot et al. 2006).

In Turkey, there is a range of mechanisms including statutory designations, national planning policies and international conventions for the protection of natural and cultural components of landscapes and wetland ecosystems. For wetlands in particular, government planning regulations provide strong measures to help support the management and protection of these ecosystems. Some of the measures are; Ramsar Convention, national wetlands regulation related with Ramsar Convention and, local and national wetland commissions. Furthermore, ELC and attempts for the implementation of ELC are also promising means for the protection of wetland landscapes in Turkey. However, these mechanisms and measures are rarely and adequately included into the regional and local planning processes during the preparation and formulation of development plans and strategies in the country. As a result, despite various attempts in international and national levels for protection and sustainability of wetland landscapes, these landscapes are under threat of current human activities as well as regional and local development plans which gives priority to economic development. This is the case especially in the less developed regions of Turkey such as eastern and south-eastern Anatolia.

The paper attempts to display the current status of wetlands in Van Province, to display the attached roles to wetlands in the local development plans and strategies and to discuss the landscape protection process with a focus on wetlands in the Province on the case of Van Basin Development Plan (2010-2013).

2. NATURAL AND CULTURAL FEATURES OF RESEARCH AREA

2.1 The geographical position

The research area Van Province is located on the Closed Basin of Lake Van, Upper Murat-Van Section and lies between longitudes 42º 40’ and 44º 30’ E and latitudes 37º 43’and 39º 26’ N (Figure 1). There are high mountains in the north and south and high plateaus in the East of the area. The west of the province is covered by Lake Van. The land area of Van is 19,069 km^2, while the whole area is 21,823 km^2 including Lake Van. Accordingly, Van comprises %2.5 of the total area of Turkey (Van, 2010). The province which is surrounded by volcanic mountains has an average altitude of approximately 2000 m. The mountains surrounding the largest part of the province constitute about the %53.4 of its area. As the second great land form, the plateaus comprise the %32.9 of the area after the mountains. The plains among the mountainous volcanic formations and fluvial environments comprise the %13.7 of the total area of the province (Alaeddinoğlu 2006).

On the shores of Lake Van, alluvium drifted by the rivers has filled the plains which have lower altitudes compared to environs (Yurt Ansiklopedisi 1985). There has been no formation of broad rivers as a result of an increasing steep slope from north to south in the topography of the west, the north-west and the south sides of Lake Van and the current rivers are short. While almost all of the rivers running constantly and temporarily during the year are discharging their water to Lake Van, Memedik Stream empties into Lake Erçek (Yurt Ansiklopedisi 1985). The main flora is steppe notably in the east side and in the west and north sides of Lake Van and in the province (Gürbüz 1994).

2.2 The socio-cultural and economic characteristics

Van had contained many cultures in the past due to its geographical position. Thus, as for natural landscape, it has many historic and cultural landscape features which are rich and various in content (Güler et al. 2011).

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%66 of the total land size of Van consists of meadows and ranges. The short periods of vegetation in Van raise the importance of an agricultural economy rather than an industrial one and put forward livestock farming in other agricultural activities because of wide meadow-range fields and high forage crop plantations. Therefore the province has the characteristics of a typical agricultural zone with regard to vegetable and animal production value (Güler et al. 2011).

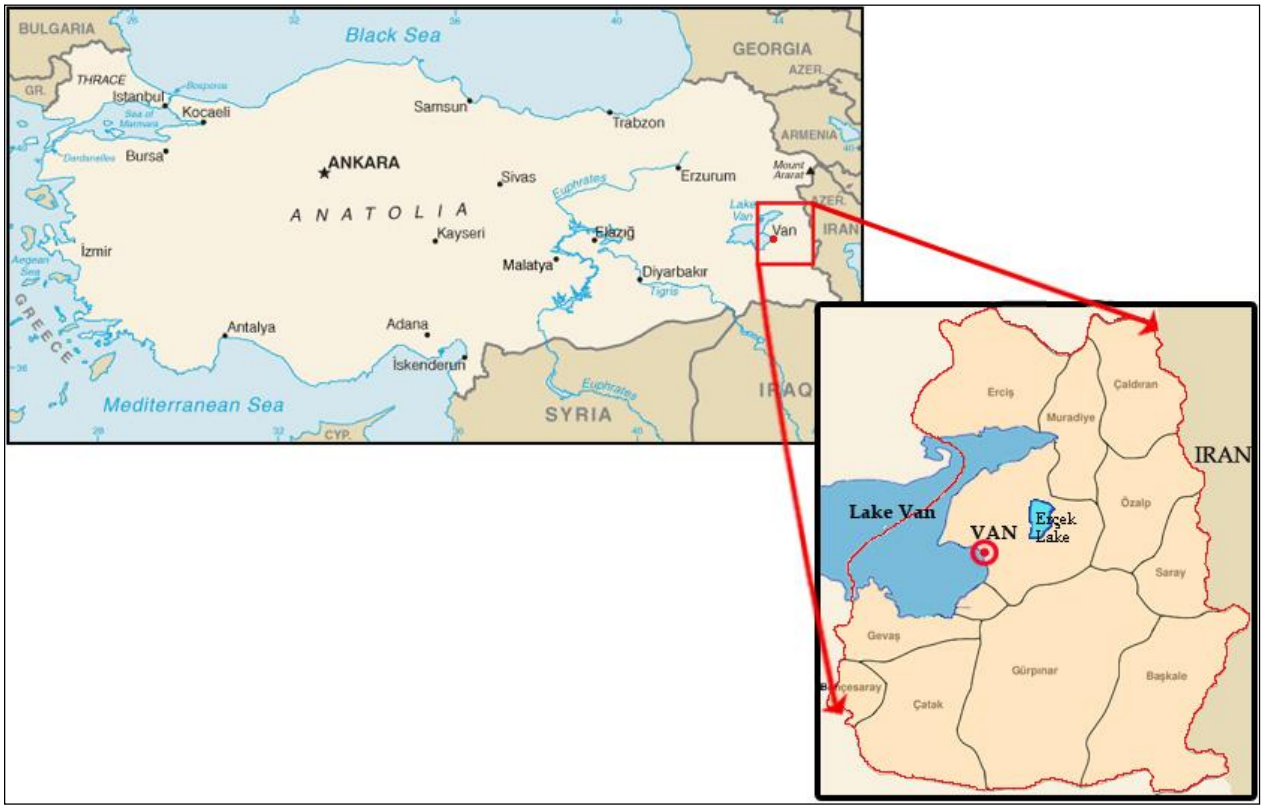

Figure 1. Geographic location of the research area

According to 2001 data of TUIK, Van is ranked as the 72th of 81 provinces in per capita GDP (Gross Domestic Product). The rate of unemployment is above the country's average with %15.6 to %14 according to TUIK data of 2009. Van is ranked as the 77th of 81 provinces in terms of the level of education and the resources of educational opportunities (Güler et al. 2011).

Van is ranked 75th among 81 provinces in terms of the development level based on the analyses of 2003. In this respect, the province is in the fifth degree developed provinces status according to the development index and there is a big gap between the country average and the province's level with regard to development and competitiveness (Güler et al. 2011).

2.3 Lake Van and its surrounding wetlands

Lake Van, which is the largest wetland in Turkey, is in the boundaries of the province of Van. Being the largest lake of Turkey with an area of 371,300 hectare square, Lake Van is like an inland sea in the middle of the high mountains surrounding it. Lake Van and its surroundings are quite rich in wetlands, especially where rivers empty into the Lake. The primary rivers which feed the Lake are Karasu, Keşiş

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Stream and Hoşap Stream in the west, Bendimahi Brook in the north-east and Zilan Stream and Deliçay Stream in the North. But, there are also many small rivers pouring into the Lake in the south, the west and partially in the northern sides of the Lake. There are not significant reeds and delta areas especially in the western and southern sides of the Lake because in these areas rivers and streams are short and don't carry enough water and material. But most of the rivers in the east, the northeast and partially in the north are leaving much of their material at the entry of the Lake and make reeds in shallow plains and small deltas. In these reeds nest many species of water fowl. While the greater part of these water fowl species are composed of migrant birds, another part is "Mid-winter Water Fowls". Of 28 species, 6433 water fowls and 1641 Lake Van gulls have been counted in the Lake which does not freeze in the winter and therefore is of rich bird diversity. Accordingly, Lake Van is considered as the most important breeding area in Turkey. The Lake Van Basin is rather close to the northeast-south migration route, which is one of the three most important routes in the West Palaearctic. The number of bird species is reported as 213 in the Lake Van Basin according to the observations and researches to date but it is expected that the number may exceed 300 with the growing ornithological researches (Adızel 1998; Durmuş 2008). Of these species, 77 are natives (%37,8), 18 are winter visitors, 89 (%43,2) are migrants and 20 (%6.1) are transit migrants. 14 of the observed species are in critically endangered, 33 are in endangered, 50 are in vulnerable, 21 are in declining, 18 are in near threatened and 25 are in least concern categories. Besides there are 13 species which are globally endangered (Durmuş et al. 2011). Consequently, some of the main areas with high potential in terms of wetland ecosystems around Lake Van and in the borders of Van Province are: Bendimahi Delta, Çelebibağı Reedbeds, Dönemeç Delta, Erçek Lake, Edremit Reedbeds (Figure 2).

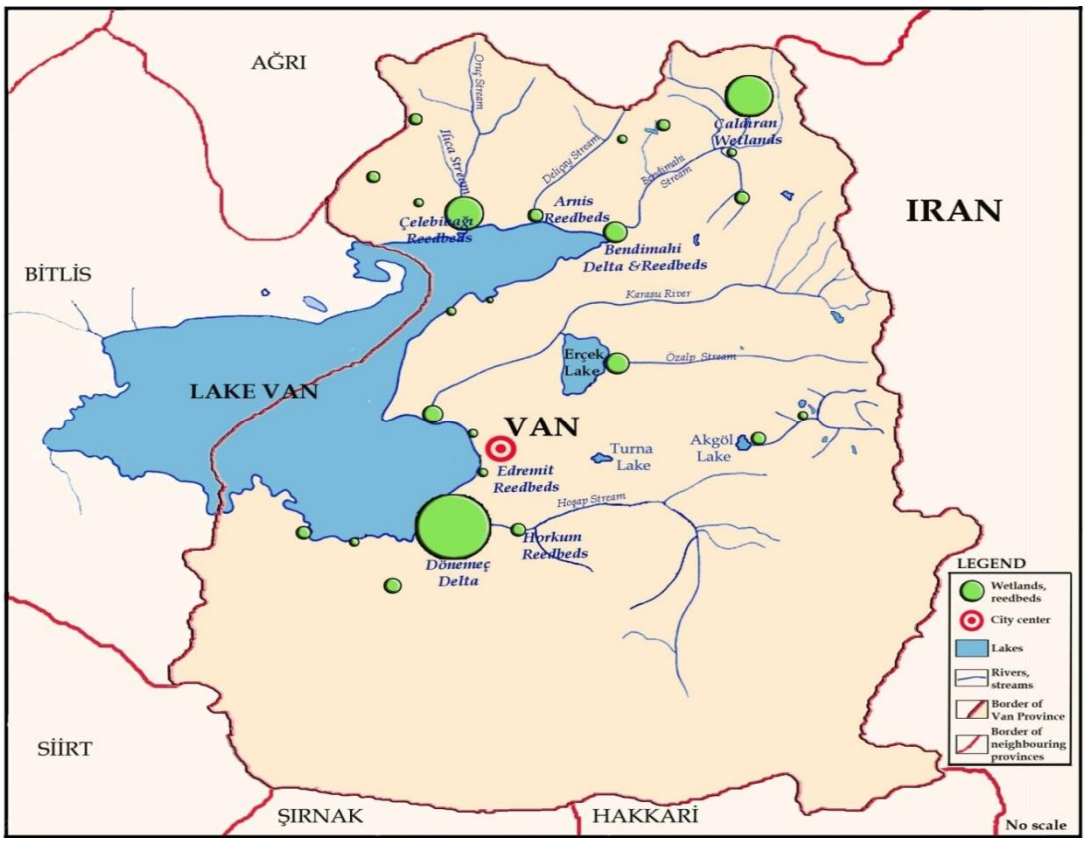

Figure 2. Natural lakes, wetlands and reedbeds in Van Province

Edremit Reed Beds: Is located in Edremit bay in the southeast of Lake Van. It is a shallow area with small lagoons, covered with reedbeds and is separated from the Van Lake by strands of sand next to Van Airlines. It is likely to see many species of water fowl in the reeds and a substantial amount of these birds breed in these reed beds (Yıldırım 1999). Many birds and water fowls visit the area in migration seasons in flocks. There are many marsh areas covered densely with small plants and reeds. Many birds choose this area because of its shallow water, its conditions of sunlight, wind and food (Z Karakoç 2013, pers.comm., 6 February). There is less number of species and amount of birds than the other wetlands in the basin as a result of the close location to Airport and settlements and, the noise of the surrounding recreational activities. Almost all of its surrounding reed areas are destructed (Z Karakoç 2013, pers.comm., 6 February).

Dönemeç Delta: It is located on the southeast shores of the Van Lake in the boundaries of Çiçekli Town, Edremit, Van. The Van- Gevaş highway divides the delta into two. The delta is covered with reeds, dunes and mud flats. Fruit growing, agriculture and livestock farming are activities on the meadows and agricultural fields. The zone has become an ideal place for many species of fish, birds and water fowls due to its good conditions for feeding, nesting and breeding. Thus, many birds and water fowl visit the area in coveys during migration periods. Many birds and waterfowl breed and stay in the area. *Marmaronetta angustirostris* (VU), *Aythya nyroca* (VU), *Phalacrocorax aritotelis*, *Larus michahellis* (NT) are some of them. The delta and its surrounding is an important breeding area for globally endangered *Oxyura leucocephala* (EN). Recent researches showed that Caspian tern (*Sterna caspia*) breeds in the area (Adızel and Durmuş 2007; Z Karakoç 2013, pers.comm., 6 February).

Dönemeç Delta is also an important river mouth for pearl mullet (*Chalcalburnus tarichi*) living in Lake Van for breeding. It is also detected that fresh water fish species *Capoeta kosswigi* live here. The area is also important for carp (*Cyprinus carpio*) (Z Karakoç 2013, pers.comm., 6 February).

Çelebibağı Reedbeds: It is a reed and mud area covering approximately 990 ha on the coast where Ilıca Stream empties into Lake Van on the southwest of Erciş district. The area hosts many waterfowl species. It is an important breeding and nesting area for many bird species, especially seagull species. Among the others *Tadorna ferruginea* (LC), *Marmaronetta angustirostris* are the IBA trigger species recorded in the area (Birdlife International 2013a). *Aythya nyroca*, *Grus grus* (VU) ve *Himantopus himantopus* (VU) are among the main bird species breeding in the area. 1.500 flamingos (*Phoenicopterus ruber* (D)) stay here for two months during their migration period. *Cygnus cygnus* and goose coveys give satisfying views for bird watchers (Z Karakoç 2013, pers.comm., 6 February). The area is also among important home ranges for pearl mullet (TC Orman ve Su İşleri Bakanlığı 2013).

There are agricultural and settlement areas in the close vicinity and thus the area is exposed to negative impacts of the human activities (Z Karakoç 2013, pers.comm., 6 February).

Bendimahi Delta: The 370 ha delta is formed on the northeast of Lake Van due to decreasing slope where Bendimahi Creek reaches to the lake. There are meadows and reeds on both sides of the creek flowing through the delta. On the mouth of the delta, where the creek connects to Lake Van, there are mud islands and reeds. Some part of Van-Erciş Highway passes through the area (Yıldırım 1999).

Bendimahi reed is a home range for many birds. Endangered species *Marmaronetta angustirostris, Aythya nyroca* and *Oxyura leucocephala* breed and feed here. *Phalacrocorax pygmeus*, *Sterna albifrons* (D) and *Gelochelidon nilotica* (NT) also leave here. High numbers of *Larus michahellis*, *Sterna caspia, Pelecanus onocrotalus* and *Grus grus* feed in Bendimahi Delta and reeds in the summer months (Z Karakoç 2013, pers.comm., 6 February).

Erçek Lake: It is an alkali lake formed in a tectonic subsidence area blocked by lava flow on the north-west on 20 km east of Lake Van. Erçek Lake is an inland wetland; with the standing fresh water and water fringe vegetation features (Birdlife International 2013b).

The deepest part is 30 metres and there is no outflow. It covers 20,380 ha and has an altitude of 1890 metres. Northern and western coasts are surrounded by escarpments, southern and eastern coasts by shallow coasts and wide mud plains. There is a small delta with some reeds and wet meadows in the location where Memedik River enters the lake on the east. Moreover, there is a small peat bed adjacent to the southeast end of the lake. There are agricultural areas and steps around the area (Z Karakoç 2013, pers.comm., 6 February).

South and the east of the lake are rich in terms of bird species. Reeds and marsh are located mostly in these areas. According to the results of ornithological observations in 1996, 180 bird species were detected in the reeds. Most of the bird species breed in this reed bed. Breeding species are: *Podiceps nigricollis* (NT), *Tachybaptus ruficollis* (D), *Egretta garzetta* (D), *Himantopus himantopus, Tringa ochropus* (EN), *Burhinus oedicnemus* (EN), *Recurvirostra avosetta* (NT), *Charadrius leschenaultia* (EN), *Podiceps grisegenea* (VU), *Oxyura leucocephala* (EN), *Charadrius alexandrinus*, *Tringa totanus* (NT) and *Acrocephalus agricola*, *Podiceps nigricollis* (NT) and *Tadorna ferruginea* (NT) are observed in significant numbers in the lake. Owners of the area are flamingos. 1,000 flamingos, which are envisaged to breed on Urumiye Lake in Iran spend summer and autumn on Erçek Lake (Yıldırım 1999).

Animal husbandry is carried out on the marshes and pastures around the lake. Tourism and bird watching activities have started to increase recently. Lake shore is also used for picnic and side seeing purposes (Z Karakoç 2013, pers.comm., 6 February).

Other than these wetlands, there are some small size wetlands on riversides in the research area and at the points where these rivers reach to Lake Van, such as Akdamar Reedbed and Horkum Reedbed. Other than reed beds and marshes, there are many small size lakes in the area. Main small lakes are Çenge Lake on 3 km west of Erçek Lake, Turna Lake, Ak Göl on the south of Ozalp district, Sor Lake on the south of Saray District, Kaz Lake on the east and Cengen Lake, 20 km south of Saray district, Sultan Lake on the east of Muradiye plateau, Hıdırmenteş Lake on the west of Çaldıran district, Kaz Lake on the north of Çaldıran, where Tendürek Mountain meets the plateau, and a couple of small lakes close to Iran border (Yıldırım 1999).

Main information obtained in the research on some wetlands in the Province is presented in Table 1. Wetlands with no available data are not given in the table.

2.4 Main problems and threats on the wetlands in the research area

Wetlands in Van Province are affected from the human activities carried out in the vicinity directly and indirectly both in the short run and the long run. Main problems and threats affecting ecological sustainability of the wetlands in the research area are as follows;

- Lack of updated and accessible data base for each of the wetlands located in the Province and near surrounding,
- Insufficient resources for environmental awareness and sensitivity in the society, among decision makers and practitioners,
- Insufficient human resources for protection and management of wetlands in the local government units,
- Insufficient knowledge among local decision makers and practitioners on the importance of wetland landscapes,

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Table 1.Some of the wetlands and their main features in Van Province (developed from Durmus and Adızel 2007; Durmus 2008).

Wetland name	Approx. height from sea level (m)	Area (ha)	Town	Conservation status (National/ International)	Management plan		Breeding, nesting red list waterfowl
					exist	not existing	
Bendimahi Delta	1700	370	Muradiye	None		x	M/VU, M/EN, M/NT, R/NT, TM/D
Caldıran wetland	2020	2000	Caldıran	None		x	M/VU, M/D, R/NT, R/D, M/VU, V+M/LC, WV/NT, WV/LC, R/LC
Celebibağı Reed beds	1750	900	Erciş	None		x	TM/D, M/VU WV/ VU
Dönemeç Delta	1662	3000		National		x	M/EN, M/VU, R/NT, M/EN
Edremit Reed beds	1650	1855	Edremit	None		x	R/NT, R/LC M/VU, M/EN, M/D, M/LC, M/NT, TM/VU, P/NT WV/EN
Erçek Lake	1803	9520	Merkez	None		x	R/EN, TM/EN, M/EN, M/VU, M/NT, R/NT, WV/NT, R/D, TM/D, WV/LC, R/LC, R/LC
Turna (Keşiş) Lake	2550	700	Merkez-Gürpınar	None		x	R/NT, R/D, M/VU,WV+M/LC, WV/NT, WV/LC, R/LC

Statues of Red Data Book for bird species;
EN: Endangered **NT:** Neat Threatened **VU** :Vulnerable **D:**Declining **LC:** Least Concern
M : Migratory **R** : Resident **WV:** Winter Visitor **TM:** Transit Migratory

- Lack of coordination among institutions and jurisdictional dispute,
- Insufficient number of solid waste storage, sewerage and waste water treatment plants in the province,
- Consideration and usage of Lake Van and other wetlands in the province as discharge areas for any kind of polluters and waste,
- Transportation of excessively used chemicals and fertilizers in the agricultural areas with surface water to the wetlands,

- Withering with the purpose of agricultural and settlement usages,
- Deposition of eroded soil in the wetlands,
- Uncontrolled hogging removal in the riverbeds,
- Unplanned and faulty reed cuts,
- Excessive grazing and weed cutting on the meadows around the wetlands,
- Illegal hunting,
- Bird egg collection in the reed beds and meadows,
- Unplanned recreational development,
- Harms given by people in recreational activities without awareness regarding features of these ecosystems and sensitive balances,
- Developments in the province and around Lake Van without a plan and infrastructure (Adızel 1998; Yıldırım 1999; Güler et al. 2011; Z Karakoç 2013, pers.comm., 6 February).

3. ROLES AND EXPECTATIONS IMPOSED ON THE WETLANDS IN VAN PROVINCE IN THE REGIONAL PLANS

1/100.000 scale Muş- Bitlis- Van Environmental Plan aims at "taking special measures for conservation of internationally important wetlands and other areas without a legal status" for the wetlands in the province. It is envisaged in the plan to consider wetlands as conservation areas of natural characters. It is mentioned that the Regulation on Conservation of Wetlands will be followed during the activities in this area. However, conservation zones for the wetlands in the province are not determined, but just ecological impact zone borders are determined. As a part of the plan, wetlands in the province are assigned the role of developing eco-tourism and the activities within this concept (Egeplan Planlama Ltd. Şti. 2011).

The other plan determining usage and conservation of wetlands in Van Province today and in the near feature is TRB2 Region 2011-2013 Regional plan. The plan covers Van district and neighbouring Bitlis, Hakkari and Muş Provinces. The plan emphasizes "…being a region in peace with the nature…" in addition to socio-economic development in the determined development vision (Güler et al. 2011). However, within the frame of targets determined for the region, conservation of wetlands and other landscapes are not handled directly. Natural landscape and wetlands are considered as sources for development of tourism sector for socio-economic development in non-agricultural sectors (Güler et al. 2011). The plan considers the wetlands as one of the natural resources that will take a place in revival of tourism. Accordingly, in wetlands in Van and other regions, the role of developing alternative tourism activities in the natural areas is assigned.

One of the targets of 2011-2013 Regional Plan, that might indirectly contribute to conservation of wetlands and other natural landscapes, is development of "environmental awareness" in the region. As a part of this target, the plan foresees "development of environmental conservation awareness for development of social life awareness" in relation to the development of human and social capital in the region (Güler et al. 2011). In addition to this target, it is aimed to improve environmental end energy infrastructure with the strategies of "provision of sustainable waste water management " and "sustainable disposal of solid and hazardous waste" within the framework of "improvement of urban and rural infrastructure" aim in the region within the planned period (Güler et al. 2011). Despite this, no indicators are determined regarding the ecological features and conditions of the wetlands and/or

other natural areas as a part of performance indicators of targets and strategies determined in the regional plan.

4. CONCLUSION AND DISCUSSION

Emphasis and priority given to landscape protection and ecosystem conservation are growing around the world. However, emphasis and priority given to these theme areas are being affected and shaped by the main approaches and policies of development plans and strategies in various scales as well as by the development levels and priorities in different regions of a country. In developed regions, it has been observed that landscape quality is regarded as among the main indicators of development so as the life quality. While landscape quality is a multi-dimensional concept, ecological sustainability and landscape protection constitute vital components of this concept. However, in less developed regions, especially in less developed rural areas sustainability of ecosystems and landscape protection are often neglected among stakeholders and in the development plans. This is also the case that wetland landscapes confronted with in Van according to the review on current status of wetland ecosystems in the province. The role, as a material for ecotourism, attached to wetlands in development plans for the Province shows that these landscapes regarded as resources to consume for economic development. Threats and problems, wetland landscapes are confronted with in Van also show and prove that these areas only important for economic value for national, regional and local stakeholders. Furthermore, among all the wetlands which are rich in biodiversity and the wetlands important for IBA species, only one of these (Dönemeç Delta) has national conservation statute but none of them is designated as a RAMSAR area. To overcome these issues for the ecological sustainability of the wetland landscapes in Van, improvement of human resources capacity and institutional capacity in local units of General Directorate of Nature Conservation and National Parks of Ministry Forest and Water Works of Turkey appears as the leading priority. Efforts in these contexts should be supported by the awareness rising activities on wetlands among local decision makers and local people as well as preparation the integrated wetland management plans. In this context, formulation of strategies and action plans which support landscape protection and economic development at the same time at the regional and local planning processes are another priority for the ecological sustainability of the wetlands in Van and near surrounding. Accordingly, wetlands in the Province should have appropriate conservation statues. Erçek Lake, Bendimahi Delta, Çelebibağ Reedbeds, Dönemeç Delta and Çaldıran Wetlands are among the areas that should have conservation statues at national level as well as at international level according to the RAMSAR Convention. Furthermore, for the protection of wetland landscapes in the Province ELC should be regarded as another guiding and supporting tool in regional and local development plans. Consequently, institutional collaboration in various scales appears as one of the main requirement during the protection of wetland landscapes in Van.

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THE INFLUENCE OF SOWING DATE ON YIELD AND QUALITY OF CONFECTIONARY SUNFLOWER

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Abstract

The sunflower beside oil production can use as confectionery for human food. This type of sunflower hybrids apart from high yield should to have good quality; it means higher protein content and mass of 1000 seeds and lower oil content in the seed. According the two year field experiment with 3 confectionery hybrids we find out that highest seed yield had hybrid NS Gricko (4,30), and was at sowing dates between April 20 and May 10. Oil content were pretty low the lowest had hybrid NS Goliat (29,55%) and were regularly decreased with later sowing dates. In 2011 protein content and seed size were higher for 2.4% and 10.68 g than in 2012. NS Gricko had the highest protein content (15.92%) and hybrid NS Goliat (105.96 g) the highest seed size. Generally protein content was higher in the middle than in early sowing dates, while 1000 seed mass was higher at early or middle sowing dates than late.

Key words: *confectionary sunflower, sowing date, yield, seed size, % protein*

1. INTRODUCTION

Sunflower is produced mainly as oil crop and its oil is one of the world's major vegetable oils. Also sunflower seed can use as confectionery for human food like snacks or like addition in the cakes and breads and also is common the use of sunflower like birdfeed or for ornamental purposes. For those non-oil purposes, only about 10% of the world sunflower production is used. Breeding target of confectionary sunflower hybrid differ from oil types, it is directed towards the increase of protein content (>25%), mass of 1000 seeds (>100 g), volume mass, oil stability with lowering oil content in the seed (<40%), increase of kernel ratio with the lowering of the shell ratio, uniformity in seed size and color, dehullness as well as tolerance to dominant diseases in the cultivation region (Hladni et al. 2011). Seed of high protein sunflower is usually black with white stripes or colorful, significantly bigger than the seed of oil type sunflower with thicker hull loosely connected to the kernel. The shell is easily separated from the kernel and allows the whole seed to be dehulled (Gonzalez-Perez & Vereijken 2007). The suitability for alternative uses of sunflower oil is determined mainly by fatty acid composition and the amount of antioxidants (Pereyra & Aguirrezábal 2007). Oxidative stability, which delays the loss of nutritional value and the development of unpleasant flavors, depends on the proportion of oleic acid (18:1) and the amount of antioxidants, mainly a tocopherol (Fernández-Cuesta et al. 2012).

In the US, seeds from confectionery cultivars are separated into three grades based on size. The largest seeds (>7.9 mm), called "in-shell seeds", are marketed unhulled, salted and roasted to be consumed as a snack food. Medium-size seeds called "hulling seeds" are hulled and the kernels are used, either roasted or not, as a snack food or in a number or confectionery or bakery products. Finally, smaller

seeds (<7.1 mm), called “bird seeds”, are mainly intended for feeding wild birds and pets (Lofgren 1997).

Sunflower is usually grown in rainfed systems because it is relatively drought tolerant (Robinson 1970, Lindstrom et al. 1982, Stone et al. 2002) and utilizes soil nutrients efficiently (Connor and Hall 1997, Valchovski 2002) due to its well-developed and deeply penetrating root system. In spite of this, soil water availability is normally rather low during flowering and seed maturation, which are crucial periods in terms of the yield potential of this crop because water deficits during these periods reduce the supply of assimilates for reproductive growth and consequently the crop yield (Cirilo & Andrade 1994, Andrade 1995).

In Mediterranean climate yield variation of rainfed sunflower is reported to be greater when sowing is delayed (Bange et al. 1997, De la Vega and Hall 2002). With delayed sowing, development is hastened because the crops encounter higher temperatures during the vegetative growth (Cirilo & Andrade 1994). Shortening of the growing cycle decreases the amount of radiation intercepted during the growing season and thus total dry matter at harvest (Andrade 1995; De la Vega & Hall 2002). Delay of the sowing date significantly reduced seed yield of sunflower due to a decrease in the number of seeds m^{-2} and in seed weight (De la Vega & Hall 2002). Also in standard oil types hybrid, late planting dates affect negatively oil yields through reductions in all its primary components i.e. seed number, seed weight and seed oil content (Robinson 1970, Unger 1980, Andrade 1995, Bange et al. 1997). According to Baros (2004) early sowing date increase seed yield. The disadvantage of low temperatures in early sowings and a consequent delay in the crop emergence is compensated by a higher probability of more favorable moisture conditions, which are even more important for a fast and uniform crop establishment. As reported by de la Vega & Hall (2002) the seed set in the central portion of the head can be used as an indicator of hybrid adaptation to late planting dates and that the hybrids that improve their relative performance at late planting dates are characterized by canopy stay green.

This paper reports the results of a 3-year study of the effects of planting date on yield and quality of domestic confectionery sunflower hybrids in the northern part of Serbia.

2. MATERIALS AND METHODS

The experiment was conducted at the experimental field Rimski Sancevi, of the Institute of Field and Vegetable Crops, Novi Sad, Serbia. The soil type was the chernozem, one of the most fertile soils. A split-plot design field experiment with four replications was conducted during the 2011 and 2012 cropping seasons. Main or large plot consisted of three sunflower hybrids: NS Goliat, NS Slatki, and NS Gricko. Certified seed of hybrids was provided by Institute of Field and Vegetable Crops, Novi Sad. Subplots were eight sowing dates, from 20 March to 1 June, within an interval of ten days. Individual experimental plots were 10 by 4,2 m, with six rows in every plot. Planting was done manually with 2-3 seed per hill; at 70 cm inter row space and 25cm in row. At stage of 2-3 pair of leaves the plots were thinned at one plant per hill. Harvesting was done manually at maturity by harvesting the four inner rows in each plot. Even there we did not harvest first and last plant in those rows, because of edge effects. Sunflower seeds were threshed on a stationary thresher and seed moisture was determined gravimetrically. Sunflower seed subsamples were further cleaned by hand to remove any broken seed or impurities. Seed yield was calculated to 11% seed moisture content. Seed oil content (%) was determined from absolutely clean and air dried seed on the NMR-analyzer. Seed protein content was determined by Kjeldahl method. Mass of 1000 seeds (g) was measured on a random sample of an absolutely clean and air dried seed. Protein yield was determined from seed yield and seed protein content (tha-1). Data were processed by ANOVA and

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The ANOVA was completed using the Mstat C software and model 'Two Factor Randomized Complete Block Design with Split Plot Combined over Locations'. The LSD test performed for the separation of means when the F-test revealed the error probability to justify the difference minor or equal 5% ($P \leq 5\%$).

3. RESULTS AND DISCUSSION

According to probability of F-test from ANOVA very significant (1%) effect on seed yield had hybrid, sowing date and their interaction, significant effect (5%) had interaction year*sowing date, while all other factors had no significant effect on seed yield. (Tab. 1)

Between two research years there is no significant difference in seed yield (4.04 and 4.14 tha^{-1}). On average for two years and eight sowing dates hybrid NS Gricko (4,30) had very significantly higher yield than two another, which had almost same yield. On average for two years and three hybrids the highest seed yields achieved at sowing dates in May 1 (4.4 tha^{-1}). Between it and adjacent dates there are no significant differences but early and late sowing dates had very significantly lower seed yields. In both research year yield ranks of hybrids were same and in 2011 there were no significant differences between any hybrids. In each sowing dates seed yield were higher in 2012 and in both year the highest seed yield achieved at sowing dates between April 20 and May 10. In 2012 differences between early dates and middle dates were smaller than in 2011. In each sowing dates seed yield of hybrid NS Gricko were higher than another two hybrid with exception in first sowing dates. Those differences were even significant in middle (4. and 5.) sowing dates. The highest seed yield of hybrid NS Goliat was later in 5. and 6. sowing dates, while on another two hybrids were earlier in 4. and 5. sowing dates (Tab.1).

Tab. 1. The influence of sowing date on seed yield of three confectionery sunflower hybrids in two year

Year: A	Hybrid: B	Sowing date: C								Av.: AC	Av.:A
		1. (20.03.)	2. (01.04.)	3. (10.04.)	4. (20.04.)	5. (01.05.)	6. (10.05.)	7. (20.05.)	8. (01.06.)		
2011	NS Goliat	4.01	3.95	3.59	3.98	4.39	4.19	4.08	3.56	3.97	4.04
	NS Slatki	3.84	3.68	4.04	4.11	4.27	4.09	4.08	3.52	3.95	
	NS Gricko	3.85	3.87	3.94	4.55	4.64	4.24	4.30	4.17	4.20	
	Average: AC	3.90	3.84	3.85	4.21	4.43	4.18	4.15	3.75		
2012	NS Goliat	4.21	3.92	4.13	3.90	4.16	4.41	3.72	2.78	3.90	4.14
	NS Slatki	4.10	4.00	3.66	4.60	4.30	4.16	4.50	3.61	4.12	
	NS Gricko	4.30	4.38	4.26	4.68	4.61	4.57	4.39	4.02	4.40	
	Average: AC	4.21	4.10	4.02	4.39	4.36	4.38	4.20	3.47	Av.: B	
BC	NS Goliat	4.11	3.94	3.86	3.94	4.27	4.30	3.90	3.17	3.94	
	NS Slatki	3.97	3.84	3.85	4.36	4.29	4.13	4.29	3.57	4.04	
	NS Gricko	4.08	4.12	4.10	4.62	4.63	4.40	4.35	4.10	4.30	
	Average: C	4.05	3.97	3.94	4.30	4.40	4.28	4.18	3.61		

Factor	A	B	C	AB	AC	AΔC	BC	BΔC	ABC	ABΔC
$LSD_{0,05}$	0.15	0.18	0.19	0.26	0.29	0.27	0.35	0.33	0.49	0.46
$LSD_{0,01}$	0.21	0.26	0.25	0.37	0.38	0.35	0.46	0.43	0.65	0.61
F-probability	0.171	0.003	<.001	0.274	0.046		0.009		0.083	

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According to probability of F-test from ANOVA very significant (<1%) effect on oil content had hybrid and sowing date, interaction of year and sowing dates and triple interaction, while all other factors and their interactions had no significant effect on oil content (Tab. 2)

Between two research years there is no significant difference in oil content (33,06 and 33,40%) and level of oil was pretty low, what is appropriate for confectionery type of sunflower. On average for two years and eight sowing dates, hybrid NS Gricko (35,42%) and NS Slatki had very significantly higher oil content than hybrid NS Goliat (29,55%). On average for two years and three hybrids the oil content regularly decreased with later sowing dates, and was the highest at first and the lowest at last sowing dates and those differences were very significant. In 2012 was the same relation between sowing dates like on average level, but in 2011 there were no any pattern sowing dates influence. In both research year and in each sowing dates hybrid NS Goliat had significantly lower oil content then another two hybrids. Regularly decreasing of oil content with delay sowing were in 2012 at all three hybrids, while in 2011 was only at hybrid NS Goliat (Tab.2).

Tab. 2. The influence of sowing date on oil content of three confectionery sunflower hybrids in two year

Year: A	Hybrid: B	Sowing date: C								Av.: AC	Av.:A
		1. (20.03.)	2. (01.04.)	3. (10.04.)	4. (20.04.)	5. (01.05.)	6. (10.05.)	7. (20.05.)	8. (01.06.)		
2011	NS Goliat	30.64	30.93	28.36	28.09	30.88	29.02	26.53	29.68	29.27	33.06
	NS Slatki	35.87	35.38	34.06	32.48	35.10	34.10	34.42	34.34	34.47	
	NS Gricko	36.91	35.68	34.52	35.12	35.23	36.08	34.96	35.04	35.44	
	Average: AC	34.47	34.00	32.31	31.90	33.74	33.06	31.97	33.02		
2012	NS Goliat	30.00	30.63	31.65	31.62	29.29	29.72	29.14	26.69	29.84	33.40
	NS Slatki	37.15	36.67	35.81	35.55	34.97	34.29	32.87	32.42	34.97	
	NS Gricko	36.63	36.51	36.77	35.83	35.91	34.91	33.49	33.16	35.40	
	Average: AC	34.59	34.60	34.74	34.33	33.39	32.97	31.83	30.76	Av.: B	
BC	NS Goliat	30.32	30.78	30.01	29.85	30.09	29.37	27.84	28.19	29.55	
	NS Slatki	36.51	36.03	34.94	34.01	35.04	34.19	33.65	33.38	34.72	
	NS Gricko	36.77	36.09	35.64	35.48	35.57	35.50	34.23	34.10	35.42	
	Average: C	34.53	34.30	33.53	33.11	33.56	33.02	31.90	31.89		

Factor	A	B	C	AB	AC	AΔC	BC	BΔC	ABC	ABΔC
$LSD_{0,05}$	0.48	0.59	0.62	0.84	0.93	0.87	1.13	1.07	1.60	1.51
$LSD_{0,01}$	0.68	0.83	0.81	1.17	1.22	1.15	1.50	1.41	2.12	1.99
F-probability	0.147	<.001	<.001	0.488	<.001		0.551		<.001	

According to probability of F-test from ANOVA very significant (< 1%) effect on protein content had all investigated factors and their interactions (year, hybrid, sowing date, double interaction

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year*hybrid, year*sowing date and hybrid*sowing date, as well as triple interaction yead*hybrid *sowing date (Tab. 3)

Tab. 3. The influence of sowing date on protein content of three confectionery sunflower hybrids in two year

Year: A	Hybrid: B	Sowing date: C								Av.: AC	Av.:A
		1. (20.03.)	2. (01.04.)	3. (10.04.)	4. (20.04.)	5. (01.05.)	6. (10.05.)	7. (20.05.)	8. (01.06.)		
2011	NS Goliat	15.52	15.05	14.91	16.20	15.34	14.97	15.05	15.52	15.32	16.54
	NS Slatki	15.71	16.17	15.48	17.56	17.05	16.96	17.00	17.77	16.71	
	NS Gricko	17.79	17.15	18.17	18.45	17.55	17.61	16.31	17.72	17.59	
	Average: AC	16.34	16.12	16.19	17.40	16.64	16.51	16.12	17.00		
2012	NS Goliat	13.71	13.54	14.20	13.63	13.01	13.74	13.72	14.66	13.78	14.17
	NS Slatki	14.08	13.36	14.42	14.25	14.51	14.54	15.28	15.41	14.48	
	NS Gricko	13.49	12.69	14.11	14.59	14.68	14.11	15.08	15.22	14.24	
	Average: AC	13.76	13.20	14.24	14.16	14.06	14.13	14.69	15.10	Av.: B	
BC	NS Goliat	14.62	14.30	14.55	14.91	14.17	14.36	14.38	15.09	14.55	
	NS Slatki	14.90	14.76	14.95	15.90	15.78	15.75	16.14	16.59	15.60	
	NS Gricko	15.64	14.92	16.14	16.52	16.12	15.86	15.69	16.47	15.92	
	Average: C	15.05	14.66	15.21	15.78	15.35	15.32	15.40	16.05		

Factor	A	B	C	AB	AC	AΔC	BC	BΔC	ABC	ABΔC
$LSD_{0,05}$	0.15	0.19	0.31	0.26	0.43	0.44	0.53	0.54	0.75	0.76
$LSD_{0,01}$	0.21	0.26	0.41	0.37	0.57	0.58	0.70	0.71	0.99	1.00
F-probability	<.001	<.001	<.001	<.001	<.001		<.001		<.001	

In 2011 was very significantly higher protein content than in 2012, difference was 2.4%. On average for two years and eight sowing dates hybrid NS Gricko (15.92) had very significantly higher protein content than NS Goliat (14,55) and significantly higher than NS Slatki. On average for two years and three hybrids statistically significant the highest protein content achieved at last sowing dates in June 1 (16.05%), and generally in the middle dates were higher than in early dates. In both research year hybrid NS Goliat had statistically significant the lowest protein content, while in 2011 hybrid NS Grico had very significantly higher protein content than NS Slatki, but in next year between them there was no significant difference. Protein content in each sowing date were very significantly higher in 2011 than in 2012 and in both year the highest protein content achieved at middle and late sowing dates. In 2011 in each sowing dates except last two protein content of hybrid NS Gricko were higher than another two hybrids and hybrid NS Slatki had higher protein content then hybrid NS Goliat in all sowing dates except the first three. In 2012 the differences between hybrids across sowing dates were lower, and were significantly lower on hybrid NS Goliat than another two hybrids only in middle and

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last sowing dates. In 2011 maximum protein content mainly in all three hybrids achieved at middle sowing dates and in 2012 in the last sowing dates (Tab.3).

According to probability of F-test from ANOVA very significant (< 1%) effect on 1000 seed mass had year, hybrid, sowing date, and double interaction year*sowing date, while the effect triple interaction(year*shybrid*sowing date) was significant on 1000 seed mass (Tab. 4)

In 2011 was very significantly higher 1000 seed mass than in 2012, difference was 10.68 g. On average for two years and eight sowing dates hybrid NS Goliat (105.96) had very significantly the highest 1000 seed mass and hybrid NS Slatki the lowest (95.73). On average for two years and three hybrids statistically significant higher 1000 seed mass achieved at early or middle sowing dates. In both research hybrids rank were like on average for two years. NS Goliat had very significantly the highest 1000 seed mass and hybrid NS Slatki the lowest. 1000 seed mass in each sowing date were very significantly higher in 2011 than in 2012 with exception of sowing dates of May1. In 2011 the highest 1000 seed mass were in early and middle sowing dates, while in 2012 there was no any regularity. In 2011 in each sowing dates 1000 seed mass of hybrid NS Goliat were statistically significant higher than another two hybrids and hybrid NS Gricko had statistically significant higher values then hybrid NS Slatki only in early three sowing dates. In 2012 the differences between hybrids across sowing dates were lower and with less regularity. In 2011 maximum 1000 seed mass mainly in all three hybrids achieved at early and middle sowing dates and in 2012 in the middle and last sowing dates (Tab.4).

Tab. 4. The influence of sowing date on 1000 seed mass of three confectionery sunflower hybrids in two year

Year: A	Hybrid: B	Sowing date: C								Av.: AC	Av.:A
		1. (20.03.)	2. (01.04.)	3. (10.04.)	4. (20.04.)	5. (01.05.)	6. (10.05.)	7. (20.05.)	8. (01.06.)		
2011	NS Goliat	117.16	116.96	114.46	111.73	109.04	114.10	109.43	102.49	111.92	106.46
	NS Slatki	105.04	105.79	98.55	104.25	99.64	102.56	98.91	96.73	101.43	
	NS Gricko	112.59	111.06	105.56	109.56	103.60	103.35	103.16	99.26	106.02	
	Average: AC	111.60	111.27	106.19	108.51	104.09	106.67	103.83	99.49		
2012	NS Goliat	97.23	100.17	91.04	93.88	108.71	96.39	105.42	107.22	100.01	95.78
	NS Slatki	91.03	92.37	86.65	93.87	96.67	84.99	88.10	86.59	90.03	
	NS Gricko	96.94	99.82	96.16	100.78	103.14	92.42	94.65	94.59	97.31	
	Average: AC	95.07	97.45	91.28	96.18	102.84	91.26	96.06	96.14	Av.: B	
BC	NS Goliat	107.20	108.56	102.75	102.80	108.87	105.25	107.42	104.86	105.96	
	NS Slatki	98.03	99.08	92.60	99.06	98.15	93.78	93.51	91.66	95.73	
	NS Gricko	104.76	105.44	100.86	105.17	103.37	97.88	98.91	96.93	101.66	
	Average: C	103.33	104.36	98.74	102.34	103.47	98.97	99.95	97.81		

Factor	A	B	C	AB	AC	AΔC	BC	BΔC	ABC	ABΔC
$LSD_{0,05}$	1.49	1.83	2.69	2.59	3.80	3.80	4.65	4.65	6.58	6.58
$LSD_{0,01}$	2.09	2.57	3.55	3.63	5.02	5.02	6.15	6.15	8.69	8.69
F-probability	<.001	<.001	<.001	0.165	<.001		0.115		0.019	

4. CONCLUSION

According the two year field experiment with 3 confectionery sunflower hybrids we find out that:

Seed yield like quantitative traits was influenced by hybrid, sowing date and their interaction, as well as interaction year*sowing date.

- In both year the highest yield achieved with hybrid NS Gricko (4,30), and at sowing dates between April 20 and May 10.
- The highest seed yield hybrid NS Goliat reach later in 5. and 6. sowing dates, while another two hybrids reach earlier in 4. and 5. sowing dates.

Oil content like qualitative traits in confectionery hybrids were pretty low, around 33 % and hybrid, sowing date, and interaction of year and sowing dates had a significant influence on it.

- In both year the lowest oil content achieved with hybrid NS Goliat (29,55%)
- Ooil content regularly decreased with later sowing dates.

Protein content like main quality traits of confectionery sunflower was influenced by all investigated factors and their interactions.

- In 2011 protein content was higher for 2.4% and in hybrid NS Gricko was the highest, difference is 1.37%.
- Generally protein content in the middle dates was higher than in early dates.
- In both year hybrid NS Goliat had the lowest protein content.

Seed size or 1000 seed mass was significantly influenced by year, hybrid, sowing date, and double interaction year*sowing date.

- In 2011 seed size was higher for 10.68 g.
- In both year hybrid NS Goliat (105.96) had the highest and hybrid NS Slatki the lowest (95.73) 1000 seed mass.
- Generally higher 1000 seed mass achieved at early or middle sowing dates.

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MECHANICAL MILKING IN DONKEYS: EFFECTS OF PULSATOR FREQUENCY ON MILK PRODUCTION

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Abstract

Mechanical milking may be considered of crucial importance in order to achieve maximum milk production in donkey. The aim of this study was to test the effects of milking pulsator frequencies on milk yield and milking efficiency. Two groups of donkeys (n. 5) at the 3rd month of lactation, milked by mechanical milker thrice a day, corresponding to milking pulsator frequency of 90 and 120 cycles/min, were considered. At the 1400 h milking the donkeys were treated with oxytocin after the milking, and then milked again. Harvested milk and milking time were recorded. The pulsator frequency of 120 cycles/min increased the average daily milk yield (P<0.01) and milk harvested per day (+12%), in comparison to 90 cycles/min. The pulsator frequency of 120 cycles/min also affected the lower milking time (P<0.05) and resulted in the greater milking efficiency (69.0 vs. 55.3%; P<0.05) in respect to the total milk stocked in the udder as total cisternal and residual milk. The ultrasound scanning of the udders showed several pockets of ducts empting directly into the teat instead of a single cisternal cavity.

INTRODUCTION

An increasing interest in recent years has been focusing on the use of donkey's milk for human consumption (Chiofalo et al., 2006; D'Alessandro et al., 2010; Martemucci and D'Alessandro, 2012) as a hypoallergenic substitute in children affected by cow's milk protein allergy or multiple food intolerance (Monti et al., 2007; Tesse et al., 2008). Moreover, donkey's milk seems to be a promising nutraceutical for aged people due its effects of up-regulation of the immune system (Tafaro et al., 2007; Amati et al., 2010).

Mechanical milking may be considered of crucial importance in improving milk production due to its prompt direct and indirect effects on productive aspects (Ambord and Bruckmaier, 2010). The storage of milk between milkings and its recovery at the time of milking is affected by the anatomy and morphology of the udder (Labussière, 1988) which varies widely among species. In donkeys, no information is available on the efficiency of milking in relation to different mechanical milking pulsator frequencies nor on the characteristics of the udder.

The aim of this study was to test in jennies the effects of milking pulsator frequencies on milk yield and milking time. The morphological and anatomical characteristics of the udders and teats by direct measurements and ultrasonographic scanning was also recorded.

MATERIALS AND METHODS

The experiment was carried out in southern Italy (southeastern Murgia, Apulia region, 40°37'20'' latitude, 60°17'44'' longitude) on a total of 26 healthy adult jennies of the Martina Franca breed.

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The animals were farmed in a semi-intensive system based on natural grazing and oats integration (2.0 – 2.5 kg/day/head).

The jennies were routinely milked by mechanical milker (42 kPa vacuum, 50:50 pulsator ratio) in the presence of the foal (D'Alessandro and Martemucci, 2012) thrice a day (at 0800, 1100 and 1400 h). The experiment lasted 4 weeks. Two groups of donkeys (n. 5) at the 3rd month of lactation, corresponding to a milking pulsator frequency of 90 and 120 cycles/min, were considered. On the last 3 days of the trial (28th – 30th d), in correspondence of the third daily milking (at 1400 h), the jennies were treated with oxytocin (40 IU i.m.) (Pitocina 10 IU/mL; Intervet Productions, Aprilia, LT, Italy) after the milking, and then milked again 5 min after the injection. Before and after the oxytocin administration, harvested milk and milking time were recorded. The milking time was recorded using a stopwatch to measure the time (seconds) from the application of the teat cup to the cessation of milk flow.

Morphological linear measurements of the udders were performed before and after milking on 16 jennies The external linear measurements were: length (the distance between the most cranial and caudal points of udder attachment at the intramammary groove); width (the distance between the widest lateral points of the udder); depth (the distance between the abdominal wall at the base of the udder and the base of the teat.

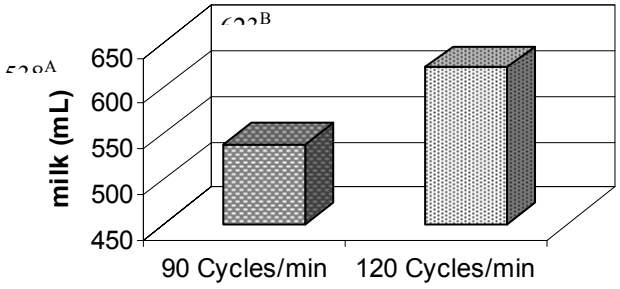
For 5 jennies, the internal structures of the udder were observed by ultrasonography with 5 / 7.5 MHz linear array linear probe (Agroscan ALR 575; Foschi, Italy) before and after milking.

All data were analysed using the GLM model for repeated measurements ANOVA (SAS 2000).

RESULTS AND DISCUSSION

The milk fraction released during the mechanical milking was considered to be 'cisternal' milk, although it is known that in the cow, a small amount of alveolar milk typically moves down into the cisternal sinus after milking (Stelwagen et al., 1996). The milking pulsator frequency of 120 cycles/min affected (P<0.01) the higher daily milk yield per milking in comparison with the frequency of 90 cycles/min (Fig. 1) which led to a significant increase of approximately 16% of the total milk harvested per day (1,869.1 vs. 1,614.1 mL of 90 cycles/min).

Fig. 1. Effect of the milking pulsator frequency on the daily milking yield per milking on 30 days.



A, B: P<0.01

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The milking time and the amount of milk recovered after oxytocin administration were significantly ($P<0.05$) lower with the milking performed at 120 cycles/min in comparison with the 90 cycles/min (Table 1).

Therefore, the frequency of 120 cycles/min resulted in a higher milking efficiency with regard to the total milk yield, consisting of the amount collected before oxytocin administration plus the amount of milk collected after oxytocin administration, in comparison with the pulsator frequency of 90 cycles/min (69.1 vs. 54.5%; $P<0.05$) (Table 1).

The improvements in the quantity of harvested milk and in milking time are in agreement with some studies in cows (Stewart and Schultz, 1958; Jorgensen and Caruolo, 1963). The amount of milk fractions after the oxytocin administration found in the jennies udders was in agreement with the residual milk amount estimated in mares (43%; Džidić, 2004) and higher than that reported in cows (10 to 30%; Bruckmaier and Blum, 1998).

Table 1. Effects of milking pulsator frequency on the means of time for milking and milk recovery before and after the oxitocin administration (means ± SD).

	PF 90 Cycles/min	PF 120 Cycles/min
Animals, n.	5	5
Before Oxytocin		
Milking time, sec	86.0 ± 12.2	84.0 ± 8.2
Milk recovery, mL	296.0 ± 52.3	488.0 ± 65.3
After Oxytocin		
Milking time, sec	77.0 ± 7.7	70.0 ± 6.5
Milk recovery, mL	354.0 ± 40.2 [a]	218.0 ± 198.7 [b]
Total milk recovery, mL [1]	650 [a]	706 [b]
Efficiency of milking, % [2]	54.5 [a]	69.1 [b]

[1]Sum of the milk recovered before and after oxytocin administration.

[2]Calculated as percentage between the milk recovered before oxytocin administration and the total milk recovery.

a, b: $P<0.05$.

In Table 2 are shown the data on the measurements of the udders before and after milking. The most prevalent shape of the udder was “bowl” in the 89% of of the jennies or “globular” in 11% of jennies. The size, shape and conformation of the udders of dairy animals are considered fundamental traits for genetic selection. In donkeys, no information is available on these aspects.

After milking, all the measurements showed a significant ($P<0.01$) decrease in comparison with the values obtained before the milking.

Table 2. Variations of the udder morphological traits in jennies measured before and after milking (means ± SD).

	Time from milking	
	Before	After
Udder length, cm	16.69 ± 1.62 ^{A}	13.77 ± 1.16 ^{B}
Udder width, cm	11.78 ± 1.83 ^{A}	9.39 ± 1.42 ^{B}
Udder depth, cm	11.19 ± 1.56 ^{A}	9.71 ± 1.62 ^{B}

A, B: $P<0.01$.

The ultrasound images of the mammary glands of the jennies showed several pockets of numerous ducts empting into the teat instead of a single cisternal cavity like in cow. Moreover, a reduction of the cisternal areas in the gland after milking was observed.

On the whole, considering the findings of this study relative to the udder anatomy and morphology, the ability of the udder to stretch between milking, and our previous study (D'Alessandro and Martemucci, 2012) we may state that jennies udder has a certain "compliance", which in cow has been related to the storage of milk (Davis et al., 1998). This explains the increase in the milk yield per milking obtained by increasing the milking interval from 3 h to 8 h, that has been attributed to the ability of the cistern to dilate (D'Alessandro and Martemucci, 2012). This is in contrast to the requirement in equids of a greater number of milkings per day with a shorter interval between the milkings (2 or 3h) (Doreau and Martuzzi, 2006) due to their low storage capability (2 L in mares) compared with ruminants (Le Du, 1986).

CONCLUSION

This study shows that in the jenny the use of milking pulsator frequency of 120 cycles/min improved mechanical milking efficiency resulting in a higher average daily milk yield per head and consequently milk harvested per day, and in lower the milking time, in comparison with the frequency of 90 cycles/min.

The ultrasound scanning of the udders showed several pockets of ducts empting directly into the teat instead of a single cisternal cavity.

The ability of the jennies's udder to stretch after milking observed in this study, confirm our previous studies (D'Alessandro and Martemucci, 2012) about the capability of mammary gland to dilate and permitting an milking interval of 8h.

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BRUCELLOSIS IN SHEEP AND GOATS IN KOSOVO

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Abstract

Sheep and goat Brucellosis in Kosovo has been reported in the last years and human brucellosis infections have mostly been in association with sheep and goat brucellosis. The aim of this study was the determination of the brucellosis immune status in three sheep herds with a small number of goats from two endemic regions in Kosovo due to high abortion rates in these herds. The possible association of sheep and goat brucellosis with human brucellosis cases from these households was aimed. The disease is endemic in Kosovo and surrounding countries. In the first herd out of 49 sheep and goats, 11 animals resulted positive using Rose Bengal Test (RBT) and Complement Fixation Test (CFT). 12 corresponding milk samples resulted positive using PCR and only one milk sample (goat) using horizontal method for isolation was confirmed as Brucella melitensis. In the second herd all of the 4 examined aborting sheep resulted brucella positive using both RBT and CFT. Out of 240 sheep in the third herd, 48 sheep resulted positive for brucellosis using RBT and 94 sheep resulted positive using CFT. This study shows the endemicity of brucellosis in Kosovo, the probably unknown brucella status of animals and the low vaccination efficacy.

Key words: animal and human brucellosis, endemic, B. *melitensis*

INTRODUCTION

Brucellosis is a global zoonosis and poses a severe public health challenge and socio-economic problems in several countries in the world. It counts as one of the major zoonotic pathogens worldwide and it is responsible for very big economic losses as well as high number of infected patients in endemic areas. The majority of cases occur in the Mediterranean countries of Europe and Africa, Middle East, India, Central Asia, Mexico and Central and South America. Four Balkan countries are considered as the most endemic countries and the region of Kosovo is not counted, where the burden of the disease has yet to be adequately quantified (Pappas, 2010). Regarding the former Yugoslavian republics a high prevalence in animals and humans was registered in the last years. Bosnia and Herzegovina counted 1639 human cases between 2011 and 2008 (Obradoviç and Veliç, 2010): in Croatia in 2008 Brucellosis was found in 370 sheep and goats and 3 cows using serology (Spicic et. al., 2010): in Serbia a prevalence of 26,8 % in sheep and 35 % in goats in 2004 was registered (Djuricic, 2006): in Bulgaria 105 cases and 635 animal cases were registered between 2005 and 2007 (Russo et al., 2010): in Macedonia 550 patients have been treated at the University clinic Skopje- Macedonia between 1998-2007 (Bosilkovski et al., 2010). The disease has been reported in the last ten years in Kosovo continuously in animals with high abortion rates and humans. In a study carried out in 7941 cattle's, 3548 sheep and 511 goats in Kosovo and the prevalence resulted 0, 58 % for cattle, 6,26 % for sheep, 7,24 % for goats (Jackson et.al., 2004). In average 30

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human cases with chronic brucellosis have been delivered in the Clinic for Infectious diseases in the last ten years (University Clinic for Infectious diseases-Kosovo, personal communication 2012), this doesn't include other cases treated in the regional hospitals and might count up to ten fold higher. In endemic areas the disease is often under-diagnosed and under-reported (Pappas et al., 2006). Although the routes of transmission have been known for decades and campaigns for eradication have been attempted in many countries and the consistently high numbers of cases indicates the challenges associated with preventing the transmission of this pathogen (Akhvlediani et. al., 2010). Some authors suggest that presently cross infections have been reported among food animals. Risk factors for humans are associated with consumption of contaminated milk and dairy products from sheep and goats and working with livestock in endemic areas (Lopes et al., 2010, Earhart et. al., 2009, Corbel 2006) direct contact with infected animal parts with open skin wounds or mucous membranes, and through the inhalation of infected aerosolized particles. An increased risk of exposition to brucellosis, the disability of the host"s defenses, the emergence of bacterial strains resistant to antibiotics and their widespread distribution as well its complexity associated with the action or inaction of man have been identified as the main factors leading to the emergence or re emergence of bacterial zoonoses (Blancou et al., 2005, Ruso et. al., 2009). In October 2011 a member of a household, a 16 years old boy after 2 weeks pain in his left leg, sweating and headache visited his ambulatory care physician and after examination he was instructed to the university orthopedic clinic and was hospitalized for 3 days. After further examinations he was transferred to the university clinic for infectious diseases. Brucellosis was confirmed using Wright test (1:1280) (Hamidi et al., 2012, unpublished data).

MATERIAL AND METHODS

Three herds consisting mainly of sheep were involved in this study. All sheep belonged to the local race DKS (Delja e Kosoves) a common sheep race in Kosovo and were of different age. All the goats belonged to the Alpina Race. The animals are held in pasture throughout the year in all the farms. 240 animals mainly sheep from the first herd, 4 sheep from the second and 49 sheep from the third herd were tested serologically after suspicions for brucellosis due to the high abortion rates ranging from 40-45 % in the first and third herd and all animals in the second herd. The first and second herds were located in the southwest Kosovo, the third herd in the southeast Kosovo. Blood samples were taken out from all of the three herds. Additionally 47 milk corresponding samples were taken for serological and molecular analysis from the third herd. RBT was performed in all samples. Positive samples were further confirmed using Complement Fixation test (CFT) according to the International Standard Methods (World Organization for Animal Health, 2009). 14 milk corresponding samples (13 sheep and 1 goat) from the first herd were tested by PCR targeting the bscp31 gene (Baily et al., 1992). PCR positive samples were cultured and typed by classical microbiological methods (Alton et al., 1988) and semi-automated biotyping system (Al-Dahouk et. al., 2010).

RESULTS

Out of 240 samples from the second herd, 48 samples (20 %) resulted positive in RBT and CFT, 46 samples were negative using RBT but positive using CFT (40 %). RBT and CFT results didn't match to each other. In The second herd out of 4 analyzed samples all animals resulted to be positive. Out of 49 sheep from the third herd using RBT and CBR, 11 samples revealed positive CFT titers (22,5 %) ranging between 1:20 (++++) and 1:160 (++++) . Results of both methods matched with each other. PCR revealed 12 brucella positive results. All the 11 samples which resulted positive using PCR were analyzed using the horizontal method for isolation of Brucella spp. and in only one revealed Brucella spp. colonies.

DISCUSSION

The results presented in this paper show that the prevalence of brucellosis is high in sheep in Kosovo despite their vaccination. The high prevalence of brucellosis in investigated herds is associated with the pastoral practices and smallholder systems as a result of different management practices, animal handling and contacts and poor control of animal movement. All sheep herds involved in this study were vaccinated with a conjuctival dose for Brucellosis in 2010 using the vaccine named Brucevac (REV 1). Sheep and goat herds seemed to have been vaccinated without knowing the immune status of the herds. In fall 2010, the same year of vaccination a high number of abortions up to 40 % was registered in the first herd, 45 % in the second herd and in all 4 sheep from the third herd involved in this study. In 2011 no vaccination was performed and the abortion rate remained high. This study shows the importance of monitoring program for Brucellosis in endemic regions and determination of the immune status of sheep and goats. 48 animals in the first herd were positive using both RBT and CFT but another 46 samples were negative using RBT but positive using CFT, proving that RBT has a low sensitivity and specificity. This confirms findings by Blascou et. al 1994, whereas the specificity and sensitivity of CFT is higher in diagnosis of sheep and goat brucellosis. Human infections were registered from the second and third herd (Hamidi, et. al., unpublished data). The infection route seems to be through animal-human contact and cheese consumption which is produced traditionally without pasteurisation. It should be aimed to identify the burden of brucellosis responsible for high persistent animal and human prevalence. Therefore, to better understand the epidemiology of Brucellosis subtyping of Brucella strains is very essential (Al Dhouk et al., 2010, 2007). MLVA -16 method will allow a better understanding of the epidemiology of Brucellosis (Gwida et al., 2012). A study in the future has to be designed to address all related factors which contribute to the persistent high animal and human prevalence of brucellosis in Kosovo.

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EFFECT OF POLYSACCHARIDES EXTRACTED FROM *PLEUROTUS OSTREATUS* AND *AGARICUS BLAZEI* ON GROWTH OF PROBIOTIC BACTERIA

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Abstrct

The effect of dehydrated basidiocarps of Agaricus blazei and Pleurotus ostreatus and their extracted polysaccharides on the growth of probiotic bacteria was evaluated. The substances were administered by gavage to male Swiss mice at concentrations of 1, 10 and 30 mg kg^{-1}. The influence of this diet on Lactobacillus spp and Bifidubacterium spp present in the caecum of the mice was evaluated. The basidiocarps of A. blazei and P. ostreatus showed no effect on growth of bacteria. The polysaccharides extracted from A. blazei stimulated in 772% the growth of Lactobacillus spp when used at a concentration of 30 mg kg^{-1}. The polysaccharides from P. ostreatus stimulated in 378% the growth of Bifidobacterium spp when used in a concentration of 10 mg kg^{-1}. The growth of Lactobacillus was also stimulated in 628% when this substance was used at 30 mg kg^{-1}. The results suggest a probiotic potencial of the polysaccharides of both fungi.

Key words: *Pleurotus ostreatus; Agaricus blazei, Lactobacillus spp, Bifidobacterium spp, probiotic.*

1 INTRODUCTION

Among the Basidiomycetes, fungi of the *Agaricus* and *Pleurotus* genera are recognized for their nutritional value, since they have high content of protein, carbohydrates and fiber and low fat content (Bonatti et al. 2004, Kalac, 2009, Rampinelli et al. 2010). Allied to the nutritional value, medicinal properties are also assigned to these fungi, such as the modulation of the immune system (Wong, Lai & Cheung 2011; Ishii et al. 2011), antitumor effect (Dalonso et al. 2009, Niu et al. 2009), antioxidant (Wong & Chye 2009) and antimicrobial (Ngai and Ng 2004, Wolff et al. 2008), antiviral, antibacterial and antiparasitic (Mizuno 1995, Pinheiro et al. 2003). These properties are provided by many bioactive molecules, especially polysaccharides (Wasser 2011) that can be found as cell wall constituents of both basidiocarp and mycelium or can be excreted to the culture medium in liquid cultures (Pramanick 2005, Synytsya et al, 2009, Smiderle et al. 2011).

Due to their nutritional and medicinal properties, in recent years, the use of fresh mushrooms or supplements based on its extracts has increased (Neyrinck et al. 2009). The beneficial properties of edible mushrooms led to the marketing of many brands based on dehydrated mushrooms in the forms of capsules and tablets to which are attributed a number of medicinal properties such as prevent and combat various diseases, including cancer, hypertension, hypercholesterolemia and diabetes, to increase or control the mental activity, to reduce the stress and to modulate the immune system. The electronic market is accomplished mainly in Brazil, Japan and China (Chen 2001). However,

according to the literature, the polysaccharides present in both fungi can act either stimulating the growth of microorganisms (Yang et al. 2008, Synytsya et al. 2009, Giannenas et al.,2011) or as antimicrobials (Bernardshaw, Hetlandy & Johnson 2005, Al Fatimi et al. 2005, Ozturk et al. 2011, Ozen et al. 2011). Thus, it becomes important assessing the effect of these products based on fungal biomass on probiotic bacteria that are part of the intestinal microbiota. This study evaluated the effect of dehydrated fruiting bodies of *Agaricus blazei* and *Pleurotus ostreatus* and their polysaccharidic extracts on probiotic bacteria present in the intestinal microbiota of mice.

2 MATERIALS AND METHODS

2.1 *Fungi*

Capsules containing fruiting bodies (basidiocarp) of dehydrated and ground *Agaricus blazei* were achieved in a store of natural food at Joinville, Santa Catarina, Brazil. The fruiting bodies of dehydrated *Pleurotus ostreatus* were obtained from the Laboratory of Cultivation of Basidiomycetes of the University of the Region of Joinville - Univille and were ground to powder in a blender.

2.2 *Extraction of polysaccharides from the basidiocarp*

The dried and ground basidiocarps of *A. blazei* and *P. ostreatus* were added to deionized water at a rate of approximately 1:13 (w/w), in order to maintain the biomass covered with water. The suspension was heated to 100ºC for 4h, by 3 times, filtered in Wathman paper No. 1 and the filtrate is concentrated in a rotary evaporator. The concentrated solution was mixed with ethanol (96%) in the rate of 1: 4 (v/v), kept under refrigeration (4° C) for 12h and centrifuged (5,000 min^{-1}, 10 min). The supernatant was discarded and the precipitate was lyophilized and kept in a sealed bottle until its use (Pokhrel & Ohga 2007).

2.3 *Animals and diet*

Female "Swiss" mice weighing 30-35g were purchased from the Technological Institute of Paraná (TECPAR). The animals were housed in groups of six animals, under standard laboratory conditions (22±1°C, 12 h light/dark cycle). After a week of acclimatization, the mice were weighed and divided into groups according to the experiment. All groups were fed a commercial diet Nuvilab CR1 and water was available ad libitum. The different evaluated substances were suspended in carboxymethylcellulose (CMC) and administered by gavage at dosages defined according to the experiment (Table 1). The control group received only CMC 0.5%. Mice received the diet once a day, for 14 days. One day after the last administration, the animals were euthanized with a pentobarbital lethal dose (60mg/kg, intraperitoneally) and a fragment of approximately 3 cm from the caecum of each animal was extracted and stored at -70°C in sterile tubes for subsequent bacteriological analysis of cecal contents. All the procedure was in accordance with the Ethics Committee on Research with Animals of the University of the Region of Joinville - Univille.

2.4 *Bacteriological analysis of cecal content*

The bacteriological analysis of cecal content was performed according to the methodology adapted from Li, Kim, Jin & Zhou (2008). The effect of the evaluated substances on intestinal microbiota of mice was assessed by the growth of *Lactobacillus* spp and *Bifidobacterium* spp using the plate count agar method. Bifidobacteria was detected using the Bifidobacterium Agar, modified (BD) containing (per liter of deionized water): Columbia Agar Base 42.5g, glucose 2.5g, lactulose 2.5g, cysteine-HCl 0.5g, riboflavin 0.01g, propionic acid 5.0 ml , agar 15g. Lactobacilli was detected using Lactobacillus Selective Agar (LBS) composed by (per liter of deionized water): pancreatic casein 10g, sodium acetate hydrate 25g, yeast extract 5g, monopotassium phosphate 6g, magnesium sulfate 0.575g,

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ammonium citrate 2g, manganese sulfate 0.12g, glucose 20g, ferrous sulfate 0.034g, polysorbate 80 1g, agar 15g and glacial acetic acid 1.32ml). The cecal contents (0.1 g) was thawed and immediately dispersed in 2 mL of a saline solution with 85% sodium chloride, 5% cysteine and deionized water, from which further dilutions were made. 25 µl of each dilution were spread on the Petri dishes containing the culture media for each micro-organism. Lactobacilli were incubated at 37°C, for 48 h, under microaerophilic and bifidobacteria were incubated at 37°C, for 72 h, under anaerobiosis. After the incubation period, plates were counted and the results were reported as colony forming units per gram of sample (CFU/g). The experiments were performed in triplicate.

Table 1 Distribution of animals according to the type and concentrations of the evaluated substances.

GROUP	SUBSTANCE	DOSE	NUMBER OF ANIMALS
Control	CMC 0.5%	0.01 mL g^{-1}	4
Ab	Dehydrated basidiocarps of *Agaricus blazei*	1 mg kg^{-1}	6
		10 mg kg^{-1}	6
		30 mg kg^{-1}	6
PAb	Polysaccharides extracted from the dehydrated basidiocarps of *Agaricus blazei*	1 mg kg^{-1}	6
		10 mg kg^{-1}	6
		30 mg kg^{-1}	6
Po	Dehydrated basidiocarps of *Pleurotus ostreatus*	1 mg kg^{-1}	6
		10 mg kg^{-1}	6
		30 mg kg^{-1}	6
PPo	Polysaccharides extracted from the dehydrated basidiocarps of *Pleurotus ostreatus*	1 mg kg^{-1}	6
		10 mg kg^{-1}	6
		30 mg kg^{-1}	6
TOTAL: 76 animals			

2.5 *Statistical analysis*

The results were analyzed by the Dixon's Q test for rejection of deviant values (Rorabacher 1991) at a 95% confidence level. The single factor ANOVA (F test) (Microsoft Office Excel 2007) was used to determine significant differences between the groups.

3 RESULTS

A negative or positive effect on growth of the tested micro-organisms were observed when the values of colony forming units (CFU) obtained from mice treated with the tested substances were statistically different from values obtained from mice treated with CMC (control).

The results of the effect of the different concentrations of Ab, Pab, Po and PPo on growth of *Lactobacillus* spp are shown in Table 2.

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Table 2 Cell growth of *Lactobacillus* spp present in the intestinal microbiota of mice treated with dehydrated basidiocarps of *A. blazei* (Ab) and *P. ostreatus* (Po) and polysaccharides extracted from the basidiocarps of *A. blazei* (PAb) and *P. ostreatus* (PPo) at concentrations of 1.0, 10 and 30mg kg^{-1}. The values represent the average of triplicates ± standard error

SUBSTANCE	CFU/g of faeces		
	1.0 mg kg^{-1}	**10.0 mg kg^{-1}**	**30.0 mg kg^{-1}**
Control	41.2 ± 18.4[a]	41.2 ± 18.4[a]	41.2 ± 18.4[a]
Ab	105.3 ±59.6[a]	109.1 ±47.4[a]	4.3 ±0.8[a]
PAb	13.6 ± 4.2[a]	207.00 ± 100.00[a]	359.3 ± 80.0[b]
Po	18.7 ±12.0[a]	214.6 ±91.3[a]	184.00 ±119.9[a]
PPo	68.2 ±31.2[a]	326.1 ±148.7[a]	300.00 ±27.0[c]

Equal letters represent no significant differences between the values obtained for each substance and the control. There is no significant difference between the different concentrations.

Results presented in Table 2 show that when mice were treated with 30 mg kg^{-1} of PAb or PPo, the growth of *Lactobacillus* spp present in cecal contents were stimulated in 772% (PAb) and 628% (PPo). The other tested substances (Ab and Po) and concentrations did not affect the growth of *Lactobacillus* spp.

Lower doses of PAb or PPo (1 and 10 mg kg^{-1}) were not enough to cause a significant effect on the lactobacilli growth. Observing that there was no effect when the whole basidiocarps of both fungi are used, and considering a yield of 0.06 g of reducing sugar per gram of basidiocarps (data not shown), it is possible to conclude that lower concentrations did not contain polysaccharides in a sufficient amount for activating the growth of the micro-organism.

The effect of Ab, Pab, Po and PPo at concentrations of 1.0, 10 and 30mg kg^{-1} on cell growth of *Bifidobacterium* spp are shown in Table 3.

Table 3 Cell growth of *Bifidobacterium* spp present in the intestinal microbiota of mice treated with dehydrated basidiocarps of *A. blazei* (Ab) and *P. ostreatus* (Po) and polysaccharides extracted from the basidiocarps of *A. blazei* (PAb) and *P. ostreatus* (PPo) at concentrations of 1.0, 10 and 30mg kg^{-1}. The values represent the average of triplicates ± standard error.

SUBSTANCE	CFU/g of faeces		
	1.0 mg kg^{-1}	**10.0 mg kg^{-1}**	**30.0 mg kg^{-1}**
Control	5.16 ±3.2[a]	5.16 ±3.2[a]	5.16 ±3.2[a]
Ab	14.4 ±8.4[a]	42.4 ±13.0[a]	13.6 ±6.8[a]
PAb	62.8 ±52.6[a]	173.6 ±126.4[a]	24.2 ±13.8[a]
Po	9.5 ±4.8[a]	302.00 ±216.5[a]	276.2 ±232.9[a]
PPo	24.9 ±18.8[a]	24.7 ±1.22[b]	35.4 ±12.2[a]

Equal letters represent no significant differences between the values obtained for each substance and the control. There is no significant difference between the different concentrations.

According to the results the growth of *Bifidobacterium* spp was activated in 378% when PPo was used at 10 mg kg^{-1}. The other substances (Ab, Pab and Po) and concentrations showed no effect on bifidobacteria growth.

Data obtained in this study showed the stimulus of *Lactobacillus* spp growth only when PAb was used at its highest dose. However, some authors report the prebiotic activity of the genus *Agaricus* in vivo for both lactobacilli and bifidobacteria. Giannenas et al. (2010) evaluated the morphology and population of intestinal bacteria in broilers fed for six weeks with different amounts of the mushroom *Agaricus bisporus*. The results showed that a diet of mushrooms did not significantly affect intestinal morphology and generally increased the population of *Bifidobacterium* spp, and *Lactobacillus* spp compared to the control diet (without mushrooms), suggesting a beneficial effect of *Agaricus bisporus*. In another study, Giannenas et al. (2011) concluded that a diet supplemented with *Agaricus bisporus* caused increase in beneficial bacteria of the intestinal microbiota - *Lactobacillus* spp and *Bifidobacterium* spp of turkeys. The diet was composed by 0, 10 or 20 g kg^{-1} of dried mushroom and was consumed as part of the normal animal feed. The dietary supplementation with 20g kg^{-1} of *A. bisporus* increased the number of *Lactobacillus* spp in ileum. In the caecum, the growth of both, *Bifidobacterium* spp and *Lactobacillus* spp, was stimulated. It can be highlighted that the dose used by this author was extremely higher than the dose used in this study.

Considering the results obtained for *P. ostreatus*, only the polysaccharides extracted from the biomass of this fungus (PPo) at a dose of 10 mg kg^{-1} were able to promote the growth of probiotic bacteria in the intestinal microbiota of mice. It is noted therefore that the prebiotic potential of *P. ostreatus* is associated to the presence of polysaccharides. Yang et al. (2008) demonstrated the effects of mannanoligosaccharides (MOS) on growth, nutrient digestibility and intestinal microflora of broiler chickens fed a diet of sorghum wheat. Two MOS levels (1 and 2 g kg^{-1}) were included in the diet and a reduction in the population of *Lactobacillus* spp and coliforms in the ileum and caecum were observed. Addition of MOS tends to reduce the load of lactobacilli and coliforms in the mucosa of the intestine. The results obtained by these authors contradict the results obtained in this work where both *Lactobacillus* spp and *Bifidobacterium* spp were stimulated in the presence of polysaccharides of *P. ostreatus*.

As in this study, other researchers have reported the growth of *Bifidobacterium* spp and *Lactobacillus* spp in animals supplemented with different polysaccharides. According to Gibson (2005) due to its chemical structure, oligosaccharides are substrates that can be consumed only by a limited number of bacteria, thus stimulating its growth. Among the group of bacteria present in the gastrointestinal tract, bifidobacteria and lactobacilli are the ones using the most oligosaccharides able to beneficially affect the host health.

Studies in vitro also report the effect of polysaccharides on probitic bacteria. In a work of Kontula et al. (1998), the effects of oat bran fermented by *Lactobacillus* GG-over activity of the intestinal microbiota, and stimulating action / inhibitory supernatant of oat bran on the growth of *Escherichia coli*, *Enterococcus faecalis*, *Lactobacillus rhamnosus* (*Lactobacillus* GG) and *Bifidobacterium lactis* subsp. *lactis* were assessed using an in vitro method. The authors used a medium containing 2.7 g (30%) and 5.6 g (60%) of oat bran added with 10 ml of fermented *Lactobacillus rhamnosus* ($10x10^8$ CFU). Results showed that the growth of *Lactobacillus* GG was stimulated by the dosage of 2.7 g (30%) of oat bran but slightly inhibited by 5.6 g (60%). Possibly, the concentration of 60% of oat bran can increase the amount of bacterial metabolites that inhibit the growth of the strain. Oat bran also favored the growth of bifidobacteria, probably due to the presence of some bifidogenic factors. Although the authors do not report which substances could be stimulating or inhibiting the growth of *Lactobacillus* GG, it is known that oat bran contains beta-glucans (Anderson & Börjesdotter 2011) similar to those found in cell walls of basidiomycetes. Su, Henriksson & Mitchell (2007) evaluated the

effect of adding eleven different carbohydrates at 1.5% concentration - fructo-oligosaccharides (FOS), inulin, arabinogalactan, soybean oligosaccharides (SOS), beta-glucan (hydrolyzate and concentrated of barley), raffinose, and glucose (positive control) to the culture medium of the probiotic bacteria *Lactobacillus acidophilus* (L10), *Bifidobacterium animalis lactis* (B94) and *Lactobacillus casei* (L26). The authors report a moderate growth of B94 when compared to the control (OD = 1.5) when beta-glucan was used. L26 had a low growth (OD = 1.0) when beta-glucan concentrate was used, that can be justified by the low solubility of this substance in the culture medium. Synytsya et al. (2009) evaluated the prebiotic effect of polysaccharides soluble in water (L1) and in alkali (L2), containing particularly β-glucans and α-glucans extracted from *Pleurotus ostre*atus and *Pleurotus eryngii*, on nine different strains of *Lactobacillus*, *Bifidobacterium* and *Enterococcus*. The results showed that in most cases, the extracts of *P. ostreatus* and *P. eryngii* promoted the growth of *Lactobacillus* being the maximal activation of approximately 70% using L1 of *P. ostreatus*. Two strains of *Bifidobacterium* had their growth stimulated for both extracts, and one strain had its growth inhibited by L1 of *P. ostreatus*. The authors conclude that these probiotic have different characteristics of growth depending on the used extract and of the specificity of each strain due to the presence of structurally diverse compounds.

The results of our work suggest a possible prebiotic potential of the polysaccharides extracted from the basidiocarp of *A blazei* and *P. ostreatus*.

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