ACETILSALICYLIC ACID ROLE IN SUNFLOWER (*HELIANTHUS SP.)* PLANT FUNGAL DISEASE RESISTANCE AND IN PHYSIOLOGICAL PROCESSES OF SUNFLOWER PLANTLETS

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Abstract

In our researches we studied the role of acetylsalicylic acid in sunflower resistance, its antifungal effect against fungal pathogens (Botrytis cinerea and Sclerotinia sclerotiorum), which has significant influence on the level and quality of the production of this plant. We studied the influence of ASA incorporated in PDA, in vitro conditions, on growing of Botrytis cinerea and Sclerotinia sclerotiorum isolated, seed germination in vitro and in vivo conditions, the effect of seed dressing on frequency of above mentioned pathogens and on sunflower yield. Moreover we studied between 2004-2009 the influence of exogenous acetylsalicylic acid (ASA) solutions administrated in different concentrations) to sunflower seeds – by presoaking it for 6 hour before germination -on some physiological and biochemical processes including: plant growth of plants, and assimilatory pigments content of the first leaves of sunflower seedlings, in comparison with the same parameters of the control lots which were treated with tape water.

Keywords: ASA, SAR, PR proteins, antifungal effect, growth, assimilatory pigments.

INTRODUCTION

Salicilyc acid (SA) and acetilsalicilyc acid (ASA) are largely used compounds in human and veterinary farmacopee (MedEx 2009, Memomed 2009). They are involved in the installation of Systemic Acquired Resistance (SAR), and play an active role in plant defense on viral, fungal and bacterian pathogens (Antofie et al.2003, Csep and Sesan, 1996).

Loake and Grant (2007) relieved that SA is synthesised by plants in response to challenge by a diverse range of phytopathogens and is essential to the establishment of both local and systemic-acquired resistance (SAR). Salicylic acid application induces accumulation of pathogenesis-related (PR) proteins. Mutations leading to either reduced SA production or impaired SA perception enhance susceptibility to avirulent and virulent pathogens. However, our knowledge of the primary signalling components activating SA biosynthesis and linking to PR proteins accumulation is rudimentary. We review progress towards characterising key players (NPR1, MPK4) and processes (methylation, amino acid conjugation, S-nitrosylation) contributing to SA-signalling and perception pathways. Further, we examine emerging data on how pathogens have evolved strategies (e.g. ABA modulation and coronatine production) to suppress SA-mediated plant defence.

Salicylic acid is an important signaling molecule involved in plant defense in both locally and systemically induced disease resistance responses. Recent advances in our understanding of plant defence signalling have revealed that plants employ a network of signal transduction pathways, some of which are independent of salicylic acid. Evidence is emerging that jasmonic acid and ethylene play key roles in these salicylic acid-independent pathways. Cross-talk between the salicylic acid-dependent and the salicylic acid-

independent pathways provides great regulatory potential for activating multiple resistance mechanisms in varying combinations (Pieterse and van Loon, 1999).
In the past two years, significant progress has been made in understanding the mechanism of salicylic-acid biosynthesis and signaling in plants. A pathway similar to that found in some bacteria synthesizes salicylic acid from chorismate via isochorismate. Salicylic-acid signaling is mediated by at least two mechanisms, one requiring the Non-Expressor of PR1 (NPR1) gene and a second that is independent of NPR1. Feedback loops involving SA modulate upstream signals. These feedback loops may provide a point for integrating developmental, environmental and other defense-associated signals, and thus fine-tune the defense responses of plants (Shah, 2003).
Moreover SA and its derivatives ASA, considered one of the new plant growth regulators, have a significant impact on the various aspects of the plant life. Gutiérrez-Coronado et al. 1998, found that SA sprayed on leaves increases significantly the root growth in soybean plants, and Gutiérrez-Rodríguez et al 1991, found that SA stimulated root growth in carrot, radish, and beet plants. Its important to know if SA stimulated root growth in ligneous species such as *Pinus patula* Schl. Et Cham, one specie extensivily planted in parks, gardens and forests of México (Perry, 1991).
However the leaves of corn and soybean treated with acetylsalicylic acid or gentisic acid exhibited no change in their chlorophyll contents (Khan et al, 2003).
Salicylic acid activated the synthesis of carotenoids, xanthophylls and the rate of de-epoxidation but decreased the level of chlorophyll pigments, both in wheat and moong plants also the ratio of chlorophyll a/b, in wheat plantlets (Moharekar et al., 2003).
Purcărea and Cachiţă, 2007, Purcărea, 2008, studying the influence of Salicylic acid (SA) on the growth of sunflower (*Helianthus sp*.), seedling roots, on their total absorbtion capacity and on content of assimilatory pigments in they primary leaves, observed that the strongest positive effect on the growth and absorption capacity of the sunflower plantlet roots was that of the SA solution of 0.1mM concentration. The chlorophyll a and chlorophyll b contents significantly increased after treatment with 0.01mM and 0.1mM SA solutions, but the carotenoid pigments content were decreased using the same concentration of SA solution.
The aim of this work was to study the possibilities of agricultural use of ASA (seed dressing) based on his antifungal effect , and to study the influence of the exogenous ASA solution (aplied by soaking the seeds for 6 hour in the solutions) on some physiological processes like plant growth and development of plants, and assimilatory pigments content of the first leaves of sunflower *(Helianthus sp.)* seedlings, in comparison with the same parameters of the control lots which were treated with water.

MATERIALS AND METHODS

Antifungal effect of ASA

Biological activity of ASA was studied in preliminary tests made in vitro and in vivo conditions. These tests were performed at the Agricutural Research Station Oradea and in the labs of the Institute for Plant Protection Bucharest, between 1993-1996 and later. The performed tests shows a promising antifungal effect of ASA produced by Sinteza Chemical Work Oradea. ASA was tested as crystals and powder concerning their effect in vitro on the growts dimension of two parasitic fungi of sunflower, *Botrytis cinerea* and *Sclerotinia sclerotiorum*. We used local isolates of these wery important parasitic fungi of the sunflower and another plants in the Western part of Romania.In the field testing we used our autohton hybrids (Fundulea 90, Felix), in natural and artificial inoculation with these pathogens.

Physiological role of ASA solutions

For study the action of ASA treatments under laboratory conditions, the sunflower seeds were soaked for 6 hours in 0.01 mM (V_1), 0.1 mM (V_2), 0.5 mM (V_3), 1.0 mM (V_4) or 5.0 mM (V_5) ASA solutions and in tap water for the control lot (V_0). Then the seeds were germinated for 7 days in plastic boxes.

The germination was made on filter paper moistened with tape water, at 20±3 °C in a Sanyo MLR 351H phytotron, day/night, and relative humidity 65-85%, under natural photon flux density. Every day, the quantity of solution from the recipients was brought to the level of 20 ml.

Growth- After 6 days we measured the length of the embrionary roots, shoots and the total length of the sunflower seedlings obtained from the seeds germination under laboratory conditions.

Assimilatory pigments - after 7 days of germination we planted the plantlets in sand, leaving them there for an additional 7 days, and sprayed their primary leaves each day with 1 ml of water.

On the 14th day we determined the content of chlorophyllian pigments of the sunflower plantlets primary leaves, using N,N-dimethylformamide, 99.9% for the extraction (Moran and Porath 1980). The use of DMF renders the process simpler and faster, since the pigments can be extracted from intact tissue. The content of the pigment was determined using a UV-visible mini–1240 Shimadzu spectrophotometer, at 664nm wave length for chlorophyll a, 647 nm for chlorophyll b and 480 nm for carotenoids. For each sample we made 3 determinations.

The data obtained after the spectrophotometrycal determination, was mathematically processed using formulae proposed by Moran and Porath 1982.

Statistical analysis - the results are averages of 3 determinations and were statistically processed using the "t- test" using *prisma 5 for windows*. The values of the probabilities were determined from tables using the values of the "t" distribution and the freedom degrees based on which the variance of the empiric series was calculated.

RESULTS AND DISCUSSION

Studying the influence of ASA on the isolates pathogen, we observed significant differences in the colony diameter depending on the used concentration. The 20mM concentration both in crystal and powder form of AAS assured significant limitation in the grows of *Botrytis cinerea* and *Sclerotinia sclerotiorum* colonies after 24 and 96 hours (table 1).

Table 1.

Influence "in vitro" of AAS incorporated in PDA the grows of *Botrytis cinerea* isolated from sunflower -colony diameter in cm-

Concentration	AAS applied			
	Crystals		Powder	
	after 24 hours	after 96 hours	after 24 hours	after 96 hours
2,5 mM	0.20 $^{++}$	7.00	0.90	2.00
5 mM	0.20^{++}	7.00	0.65^{o}	1.25^{ooo}
10mM	0.20^{++}	7.00	0.45^{oo}	1.10^{ooo}
20mM	0.10^{oo}	6.00^{oo}	0.40^{oo}	1.00^{ooo}
Check	0.15	7.00	1.00	2.00
SD 5%	0.03	0.09	0.31	0.24
SD 1%	0.04	0.14	0.43	0.33
SD 0,1%	0.06	0.19	0.61	0.47

Table 2.

Influence "in vitro" of AAS incorporated in PDA on growing of *Sclerotinia sclerotiorum* isolated from sunflower -colony diameter in cm.

Concentration	AAS applied			
	Crystals		Powder	
	after 24 hours	after 96 hours	after 24 hours	after 96 hours
2,5 mM	0.700	7.000	0.250 ooo	2.000 ooo
5 mM	0.700	7.000	0.325 ooo	0.650 ooo
10mM	0.700	7.000	0.125 ooo	0.525 ooo
20mM	0.700	5.275 ooo	0.000 ooo	0.250 ooo
Check	0.700	7.000	2.000	3.000
SD 5%	-	0.25	0.22	0.24
SD 1%	-	0.35	0.31	0.34
SD 0,1%	-	0.49	0.44	0.48

Studies regarding the influence of AAS on seed germination shows a positive effect of AAS powder applied in sunflower seed dressing on the percentage of seed germination and frequency of infected plants, after a 7 day incubation period (table 3).

Table 3.

Influence of AAS applied in seed dressing on seed germination and frecquency of *Botytis cinerea* and *Sclerotinia sclerotiorum* infection in sunflower

Seed treatment	Dose g/kg	% seed germination	F% infected plats	
			S. sclerotiorum	B. cinerea
Metoben 70WP	2	95.5	1.0	2.0
Rovral 50WP	2	95.0	1.0	1.5
AAS powder	1	93.5	2.0	3.5
Check (untreated)	-	89.5	3.5	8.5

- 7 day incubation in humid chamber

Studying the influence of ASA applied in seed dressing performed in the experimental plots of ARS Oradea, shows the positive influence on seed emergency percentage in field conditions, in comparison with the untreated check and two fungicides (Metoben 70 WP and Rovral 50 WP) usually used in seed dressing of sunflower. There was applied artificial inoculation at the sowing using mycelium and sclerotia of the fungus grows on autoclaved barley seed. Our results indicated also a limitative effect on the frequency of infected plants in comparison with the untreated check. The yield difference registered in these conditions (150 kg/ha, respective 10.3%) after the seed treatment with ASA powder (1 g/kg seed) shows the positive but not significant effect of ASA in the assured high infection pressure (table 4). and the necessity of the extension of our research in this direction.

Table 4.

Influence of AAS applied in seed dressing on frecquency of *Sclerotinia sclerotiorum* infection and yield level in sunflower- artificial inoculation with mycelium and sclerotia grows on autoclawed barley seed-

Seed treatment	Dose g/kg	% of emergence	F% infected plats S. sclerotiorum	Yield kg/ha	Differences	
					kg/ha	%
Metoben 70WP	2	95.5	1.0	1750	+300++	20.7
Rovral 50WP	2	95.0	1.0	1700	+250++	17.2
AAS powder	1	93.5	2.0	1600	+150	10.3
Check (untreated)	-	89.5	3.5	1450	-	-
				SD 5%	180 kg/ha	
				SD 1%	245 kg/ha	
				SD 0.1%	350 kg/ha	

The repeated field test in condition of natural infection confirms the favorable effect of seed dressing on the plant emergency percentage, on the frequency of natural infected plants and also on the yield quantity. The yield difference registered in comparison with the untreated

check in this case was significant (table 5). These promising positive results motivates the necessity of the extension of our research in this direction.

Table 5.

Influence of ASA applied in seed dressing on frecquency of *Sclerotinia sclerotiorum* infection and yield level in sunflower.

Seed treatment	Dose g/kg	% of emergence	F% infected plats S. sclerotiorum	Yield kg/ha	Differences	
					kg/ha	%
Metoben 70WP	2	95.7	0.5	2150	+200++	10.2
Rovral 50WP	2	96.5	0.5	2270	+220++	11.3
AAS powder	1	94.5	0.7	2100	+150++	7.7
Check (untreated)	-	90.5	1.9	1950	-	-
				SD 5%	105 kg/ha	
				SD 1%	145 kg/ha	
				SD 0.1%	225 kg/ha	

Studying the growth of the embrionary roots of the sunflower seedlings obtained from the seeds germination under laboratory conditions, after 6 days of germination, we observed that the influence of the exogenous ASA treatments was dependent on the concentration which was used (table 6).

Table 6

Estimative mean values for the length of the sunflower seedling roots, shoots and total lenghts observed in the 6-th days of seeds germination on a filter paper moistened with tap water after 6 hour soaking for the control lot in water (V_0) and in ASA solutions of different concentrations.

Biometrics / Statistic evaluation	Roots lenght (mm)	Shoots lenght (mm)	Plants lenght (mm)
Type V_0 (water)			
M ± sd	50±2,21	80±2,2	130±3,2
VC	4.42	2.77	2.46
Type V_1 (0.01 mM ASA)			
M ± sd	67±2.67	65±1.75	132±2.9
VC	3.98	2.69	0.21
Statistical signification	**	**	ns
Type V_2 (0.1 mM ASA)			
M ± sd	90±3.15	92±3.65	182±4.5
VC	3.5	3.96	2.47
Statistical signification	***	*	***
Type V_3 (0.5 mM ASA)			
M ± sd	82±2.85	90±3.61	172±3.9
VC	3.47	4.01	2.26
Statistical signification	***	*	***
Type V_4 (1.0 mM ASA)			
M ± sd	73±2.98	89±4.5	162±3.8
VC	4.08	5.05	2.34
Statistical signification	***	*	***
Type V_5 (5.0 mM ASA)			
M ± sd	22±0.98	18±0.656	40±1.2
VC	4.45	3.64	3.0
Statistical signification	***	***	***

M =mean value; st = standard deviation; VC= variability
p>0.05= nonsignificant; p<0.05 * significant; p<0.01=** distinctly significant; p<0.001=*** very significant in comparison with control lot.

In comparison with the embrionary roots of control lot considered 100% (table 6, figure 1) a very significant increase of the roots length was observed in the first 6 days of

germination, when it was used 0.1; 0.5; 1.0 mM ASA solution (the increases were between 46% and 80%). We find a distinctly significant increase of it, with 34%, from the control lot after 6 hours of sunflower preasoking in 0.01 mM ASA solution. For the treatement with 5.0 mM ASA solution it was registered a very significant decrease of roots length, with 56.0% from control lot considered 100%.
For the shoots lenghts it was observed a significant increase (between 11.2% and 15%) after preasoking the sunflower seeds in 0.1; 0.5 and 1.0 mM ASA solutions. At the variant of a 0.01 mM or 5.0 mM ASA solution treatment, after 6 day of germination the shoots length decreased distinctly significant, with 18.7%. The 5.0 mM ASA treatement determined a very significantly decrease of shoot length in comparison with the control lot.
For the total length of the sunflower seedlings after 6-th day of germination we registered a very significant increase in the case of treatement with 0.1; 0.5 or 1.0 mM ASA solutions; a nonsignificantly decrease for treatement with 0.01 mM ASA solution and a very significantly decrease for treatement with 5.0 mM ASA solution.
Studying the *content of chlorophyllian pigment (chlorophyll a and b) and carotenoids* on the primary leaves of the sunflower seedling obtained from each experimental variant, we observed that the influence of the exogenous ASA solutions treatment was dependent on the concentration which was used. The results obtained were presented in table 7 and graphically represented in figure 2.
The content of chlorophyll a increased non-significantly (with 5.3% from control lot considered 100%) after seeds presoaking in 0.01 mM ASA solution. A significant increase of chlorophyll a contents, with 12.5% from the control lot, was observed in the case of treatment with 0.1 mM ASA solution. For higher concentrations than 0.1 mM the chlorophyll a content decreased significantly and very significantly from the control lot.

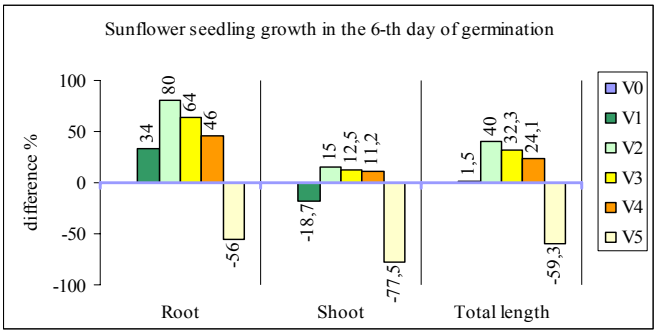

Fig. 1 – Percentage differences of the root, shoot, total length of sunflower seedlings obtained from seeds germination after soaking it in 0.01 mM, 0.1 mM; 0.5 mM; 1.0 mM and 5.0 mM concentration ASA solution, in comparison with the same parameter measured in seedling from the control lot soaked in water. The value for the control lot was considered 100%.

In the case of the chlorophyll b contents a nonsignificant increase could be observed, with 5.1% from control lot when using a 0.01 mM ASA solution, and a very significant increase, with 17.4 % from the control lot, in the case of treatment with 0.1 mM ASA solution. 0.5 mM, 1.0 mM or 5.0 mM ASA solutions significantly or very significantly decreased the chlorophyll b contents in primary leaves of the sunflower plantlets.

Table 7.

Estimative mean values for the assimilatory pigments content of the sunflower seedling leaves after treatment with ASA solutions of different concentrations.

Paramenters	V_0	Acetylsalicylic acid solutions				
		V_1	V_2	V_3	V_4	V_5
		Avarage ± standard deviation				
Total chlorophyllian pigments mg/g	0.629±0.011	0.692±0.005 *	0.72±0.003 ***	0.557±0.008 ***	0.468±0.006 ***	0.304±0.003 ***
Chlorophyll a mg/g	0.376±0.04	0.396±0.01 ns	0.423±0.003 *	0.334±0.006 *	0.281±0.009 **	0.145±0.002 ***
Chlorophyll b mg/g	0.253±0.002	0.266±0.001 ns	0.297±0.003 ***	0.223±0.01 *	0.187±0.003 ***	0.159±0.005 ***
Carotenoid pigments mg/g	0.128±0.005	0.168±0.004 ***	0.142±0.002 *	0.118±0.006 ns	0.100±0.006 **	0.07±0.001 ***

P>0.05= non-significant; p<0.05= * significant; p<0.01=** distinctly significant; p<0.001=*** very significant in comparison with the control lot

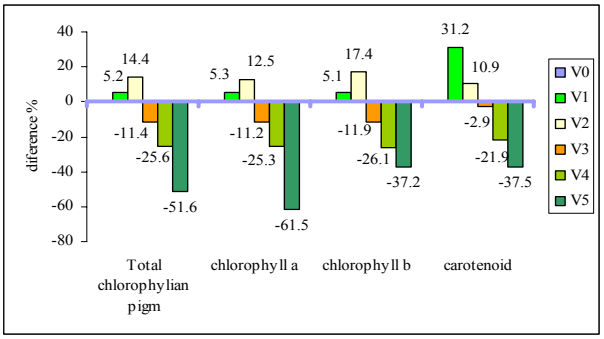

Fig. 2. Percentage differences of the content of assimilatory pigments in the primary leaves of sunflower (*Helianthus sp.)* seedlings obtained from seeds germinated on filter paper moistened with water, at 20±3°C.

The sunflower seedlings were planted for an additional 7 days in sand and their primary leaves were sprayed with 0.01 mM, 0.1 mM, 0.5 mM, 1.0 mM or 5.0 mM concentration ASA solution in comparison with the same parameter measured in the leaves of sunflower plantlets from the control lot sprayed with water. The value for the control lot was considered 100% (marked with 0 on the graphic).

Studying the carotenoids pigments content in the case of treatment with 0.01 mM concentrations ASA solution, the results show that the accumulation of these pigments in the leaves of sunflower seedling on the 14^{th} day of germination, increased very significantly, with 31.2%, in comparison with the same parameter determined from the control lot. The treatment with 0.1 mM ASA solution significantly increased this pigment contents, with 10.9%, from control lot. After treatment with 0.5 mM, 1.0 mM and 5.0 mM ASA solution significantly, distinct significantly or very significantly decreased the carotenoid pigment contents, with values between 2.9% and 37.5% from the control lot.

CONCLUSION

- The presence of ASA in PDA nutrient medium assured significant

limitative effect in the colony diameter of two pathogenic fungi (*Botrytis cinerea* and *Sclerotinia sclerotiorum*), this effect depending on the used concentration.

- ASA applied in sunflower seed dressing performed in the field plots

of ARS Oradea, shows the positive influence on seed emergency percentage, limiting the level of infected plants with *Sclerotinia sclerotiorum* in field conditions, in comparison with the untreated check and two large used fungicides. In condition of natural infection seed treatment had significant effect also on the yield level.

- The exogenous 0.01 mM, 0.1 mM, 0.5 mM and 1.0 mM ASA

solutions enhanced the growth of the sunflower seedling but any concentrations above these values proved to have an inhibitory effect. Similar results was obtained by Fariduddin et al. (2003) when they are studing the effect of exogenous SA and its derivatives on growth of *Brassica junceea* observed maximum increase in dry matter accumulation at a concentration of 0,01 mM SA, supplemented to the leaves of the standing plants of *Brassica juncea,* but any concentration higher than this proved to have an inhibitory effect.

- Diluted ASA solutions, with 0.01 mM the 0.1 mM concentration,

determined an increase in the total chlorophyllian and carotenoid pigments content in the primary leaves of sunflower plantlets especially for 0.01 mM the 0.1 mM concentration. Higher concentrations than 0.5 mM decreased the same parameter, the greatest inhibitions being obtained for the ASA solutions of a 5.0 mM concentration. Hayat et al (2005) soaking the grains of wheat in 10^{-5}m of SA solution resulted in higher pigment contents in the plants which declined as the concentration of SA was increased above that concentration. Moreover, 30 days old plants of *Brassica Juncea* sprayed with 10^{-5}M of SA solution possessed chlorophyll 20% higher than those sprayed by water only.

- Comparing the effects of different concentration ASA solutions it

was observed that on the 6th day of germination the diluted ASA solutions, with 0.1 and 0.5 mM concentrations had greater effects, the highest increase was registered for 0.1 mM ASA solution, for the majority of the parameters.

REFERENCES

1. Antofie M.M.,Carasan M.E.,BA., 2003, Acidul salicilic – moleculă semnal cu implicaţii majore în procesele de dezvoltare ale plantelor.Analele SNBC.,Vol.VIII, Cap.V.p.285-297.
2. Csep N.,Sesan T., 1996, Research on the antifungal activity of acidum acetylsalicylicum. Analele Universitatii din Oradea, Agricultură-Silicultură, ISSN1224-6263, II,p.37-51.
3. Fariduddin Q., Hayat S, Ahmad A., 2003, Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in *Brasssica juncea,* Photosynthetica 41:281-284.
4. Gutiérrez-Coronado M.A., Trejo-López C. and A. Larqué-Saavedra, 1998, Effects of salicylic acid on the growth of roots and shoots in soybean, Plant Physiol. Biochem., 36 (8): 563-565.
5. Gutiérrez-Rodríguez M., Aristeo C. P., San Miguel Ch. R., A. Larqué-Saavedra, 1991, Stimulation of root growth by salicylic acid in carrot, radish and table beet. In: Abstracts of XVI International Botanical Congress. St. Louis, USA. pp. 652.
6. Hayat, S., Fariduddin, Q., Ali, B., Ahmad, A., 2005, Effects of salicylic acid on growth and enzymes activities of wheat seedlings, Acta.Agron.Hung., 53: 433-437.
7. Khan W., Prithiviraj B, Smith DL, 2003, Photosynthetic responses of corn and soybean to foliar application of salicylates, Journal of plant physiology, 160, 485-492.
8. Loake G., Grant M., 2007, Salicylic acid in plant defence--the players and protagonists. Curr Opin Plant Biol. 2007 Oct;10(5):466-72. Epub.
9. Moharekar ST., Lokhande SD, Hara, T., Tanaka, R., Tanaka, A., and Chavan PD., 2003, Effect of salicylic acid on clorophyll and carotenoid contents of wheat and moong caryopsislings, Photosynthetica, 41, 315-317, 2003.
10. Moran R, Porath D, 1980, Clorophyll determination in intact tissue using N,N- dimethylformamide, Plant physiol, 65: 478-479.
11. Moran R., 1982, Formulae for determination of chlorophyllous pigments extracted with N,N- dimethylformamide, Plant physiol. 69 (6): 1376-1381.
12. Perry, J. P., 1991: The pine of Mexico and Central America, Timber Press, Inc. USA. pp.231.
13. Pieterse C.M.J.; van Loon L.C., 1999, Salicylic acid-independent plant defence pathways. Trends in Plant Science, Volume 4, Issue 2, 52-58.
14. Purcărea C. 2008, Studies about the influence of salicylic acid on growth and absorption capacity of Sunflower *(Helianthus sp)* plantlet roots. Buletinul USAMV-CN,65(1); pp. 390, 2008
15. Purcărea C.**,** Cachiţă-Cosma D., 2007, Studies on the influence of salicylic acid on the assimilatory pigments contents of the leaves of sunflower *(Helianthus annuus) seedlings.* Buletinul USAMV-CN, 1454-2382.pp. 356
16. Shah J., 2003, The salicylic acid loop in plant defense. Curr Opin Plant Biol. 2003 Aug;6(4):365-71.
17. x x x MedEx 2009, Medicamente explicate 2009, Ed.Medicart.
18. x x x MEMOMED 2009, Editia 15, Editura Minesan