Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454

Vol. 21 | No. 4 | pp. 1575-1585 | 2022

Original Research Paper

https://doi.org/10.46488/NEPT.2022.v21i04.010

Open Access Journal

Enhancement of Xanthan Biosynthesis Using Medicinal Herbs - A Novel Approach

B. S. Rajyaguru*, A. Varma*, A. C. Kharkwal*† and J. Singh**
*Department of Microbiology, Amity Institute of Microbial Technology, Amity University, Noida-201313, India
**Department of Global Regulatory Affairs, Danisco (India) Private Limited, Gurgaon-122002, India
†Corresponding author: A. C. Kharkwal; ackharkwal@amity.edu

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 06-01-2022
Revised: 20-02-2022
Accepted: 24-02-2022

Key Words:
Herbs
Molasses
Polysaccharide
Xanthan
Xanthomonas campestris

ABSTRACT

This study aimed to evaluate the potential of five medicinal herbs in the enhancement of xanthan gum production when used against indigenously isolated (from molasses, an agricultural waste) phytopathogen *Xanthomonas campestris* MW741556. Antibiotic susceptibility of five medicinal herbs (*Moringa oleifera*, *Bacopa monnieri*, *Glycyrrhiza glabra, Withania somnifera,* and *Arthrospira platensis*) against *X. campestris* culture was first checked. All five herbs exhibited a clear zone of inhibition against *X. campestris* during the investigation. Thereafter their effect on enhancing the xanthan gum production was studied using molasses enriched medium. The results of this experiment showed that all five herbs were capable of enhancing xanthan gum production significantly. Xanthan gum produced differed in viscosity and dried biomass. Among all, *A. platensis* and *M. oleifera* were found to be the most promising for xanthan gum production with higher viscosity. These results were further confirmed by the characterization of xanthan gum produced by five herbs using Fourier Transform Infrared Spectroscopy. Further, a multivariate approach using principal component analysis confirmed the variability among the herbs used. This versatility of these medicinal herbs opens the possibility of their utilization and application in various fields.

INTRODUCTION

Xanthan is a polysaccharide, which is a widely used food additive produced by bacteria commonly known as *X. campestris* (Petri 2015). It acts as an effective thickening agent and stabilizer in largely consumed products produced by various industrial sectors like the food industry, cosmetics, pharmaceutical, textile, petroleum, etc. (Lopes et al. 2015, Petri 2015). Based on its non–toxic and non-sensitizing nature, it has been approved as a food additive by the States Food and Drug Administration (FDA) (Kennedy et al. 1984). In food industries, it is used as a suspending and thickening agent for butter, ice creams, chocolates, etc. (Infee Sharley & Priyadarshini 2015, Suput et al. 2015). Due to its easy pourability and ability to keep the dressing on the tops of the salad, it is widely used in salad dressings also (Abedinzadeh et al. 2016).

The bacterium, *X. campestris,* is gram-negative, and aerobic, hence needs oxygen for its growth and production of xanthan gum (Sarbatly & England 2016). The conventional ways of increasing the production of xanthan in industries by *Xanthomonas campestris* on the appropriate medium under optimal conditions include the addition of sucrose or glucose as a carbon source (Garcia-Ochoa et al. 2000, Lopes et al. 2015). However, these methods are relatively expensive and increase the cost of the product produced. Researchers are more focused on exploring different waste residues which can be used as carbon sources. In addition, isolation of new strains to produce xanthan, which can metabolize different carbon sources (Bajic et al. 2014, 2015). This type of approach will not only encourage the reuse of the residual waste but will also reduce the production cost of the xanthan gum (Dos Santos et al. 2016, Ng et al. 2020).

In such a scenario, molasses, a waste byproduct of sugarcane can be an interesting option to use as a substrate for xanthan gum production, since it contains approximately 40% sucrose (Clarke 2003) hence a good source of carbon (Souw & Demain 1979) which enhances the growth of the *X. campestris* and therefore, resulting in xanthan gum production. Besides this, it is a renewable source and a by-product that is produced in large quantities during the processing of sugarcane juice. The present study also aims to evaluate the contribution of five different medicinal herbs in the production of xanthan gum along with their antibacterial effect against *Xanthomonas campestris* grown in molasses solution.

MATERIALS AND METHODS

Organism and Selection of Medicinal Plants

In this study, *in vitro* isolation of the bacterium was carried out, which was further identified as *Xanthomonas*

MW741556 using 16S_rRNA by National Center for Biotechnology Information (NCBI). Five medicinal plants (Table 1) were selected for the study based on their antibacterial properties against phytopathogens like *X. campestris*.

Inoculum for Antibiotic Susceptibility Test (AST)

The sub-cultured *X. campestris* strain was inoculated in a nutrient broth medium and incubated at 37°C for 24 h to obtain a fresh 24-hour culture. The culture was standardized according to the 0.5 McFarland Standard to obtain a standard inoculum size of $1.5x10^8$ CFU.mL^{-1}) (Magaldi et al. 2004). This standardized strain has been used as an inoculum for AST.

Sample and Standard Drug Preparation for AST

For this, 5 medicinal herbs extracts have been dissolved in methanol in four concentrations of the sample as100 µg.mL^{-1}, and 250 µg.mL^{-1}, 500 µg.mL^{-1} and1000 µg.mL^{-1}. The standard antibiotic drug Kanamycin of concentration 30 µg.mL^{-1} is also prepared which is used as a control in the experiments.

Well, Diffusion-Inoculation and Incubation for AST

Five different plates containing Mueller-Hinton agar were prepared and swabbed with the standard inoculum of *X. campestris*. After inoculation, 5 wells of 10 mm were bored in each plate using a good borer. Each plate was inoculated with 4 different concentrations of each plant sample and 1 well using the standard drug Kanamycin. Post-inoculation, the plates were incubated at 37°C for 24 h in an upright position.

Xanthan Gum Extraction

Xanthan gum production by *X.campestris* has been carried out using Molasses enriched growth media along with the addition of indigenous medicinal herbs as an additive and the effect of herbs on the quantity of the xanthan gum production was studied.

Plant Extract Preparation for Xanthan Gum Production

Five different medicinal plants 1A, 2B, 3C, 4D, and 5E (Table 1) were shade dried for a period of ten days. Then the dried plant material was grounded to a fine powder and was sieved (sieve 60 size) to obtain granules of the same size. 10 g of each sample was taken and solubilized using 10 ml of the respective solvents (distilled water and methanol) using a cyclomixer. These solubilized samples were then filtered thoroughly using a double-layered muslin cloth. This filtrate was then centrifuged at 4000 g for a period of 3 minutes. Post-centrifugation, the supernatant was collected and filtered using Whatman filter paper (No. 1). This filtrate was then heat sterilized using an autoclave at 121°C for 15 minutes and preserved in amber glass bottles.

Media Preparation for Xanthan Gum Production

X. campestris was further inoculated in molasses-rich media for the production of xanthan gum. The medium (g/l) consisted of molasses (8%), KH_2PO_4, $MgSO_4$, $(NH_4)_2SO_4$, and $CaCO_3$. Except for molasses, the remaining components of the media were autoclaved for sterilization whereas molasses was membrane filtered using a 0.45µm filter.

Inoculum Preparation for Xanthan Gum Production

The previously prepared inoculum of *X. campestris* was inoculated in 50ml of production media and incubated at 30°C for 48hours under shaking conditions of 200 RPM. The grown cultures are either used directly for further tests or preserved at 4°C until further use. This microbial culture is used as a starter culture for further tests involving xanthan gum production.

Xanthan Gum Production

The 48 hrs culture grown in the production media was added with 10% (v/v) of the respective plant extract. For the 5 herbs, 5 different media containing each specific plant extract is prepared in 5 replicates (50 mL production media with 5 g of respective herb extract). The pH of the media was maintained at 7. The inoculated media were incubated at 30°C for 48 hours under shaking conditions of 200 RPM.

Xanthan Gum Recovery

Post-incubation, the cultures were subjected to pretreatment by heating using a water bath at 90 °C for 15 minutes and

Table 1: List of medicinal herbs used in study.

Sample	Common Name	Scientific Name	Family	Parts Used
A1	Moringa	*Moringa oleifera*	Moringaceae	Leaves
B2	Brahmi	*Bacopa monnieri*	Plantains	Leaves
C3	Yastimadhu	*Glycyrrhiza glabra*	Legumes	Roots
D4	Ashwagandha	*Withania somnifera*	Solanacea	Roots
E5	Spirulina	*Arthrospira platensis*	Phormidiaceae	Algae

then filtered using Whatman No. 1 filter paper. The filtrate was then added with 50% ice-cold isopropanol (2x volume of filtrate) to which 1% (w/v) of potassium chloride was also added to stimulate the process of precipitating the xanthan gum (Kawahara & Obata 1979, Stredansky & Conti 1999). The product was then dried in the oven at 45°C for 24 h. Freeze drying is carried out to obtain the dry weight of precipitated xanthan gum. The dried biomass of cells and xanthan gum production was weighed and compared. 1% of xanthan gum solution is prepared in water and analyzed for viscosity using Brookfield Viscometer at 25°C (Dogan et al. 2007).

Spectroscopy of Fourier Transform Infrared (FTIR)

Samples of control and all five herbs producing xanthan were characterized using a Jasco Fourier Transform Infrared Spectrometer. Fourier transforms infrared spectra generated by the absorption of electromagnetic radiation in the frequency range of 400 to 4000 cm^{-1}. Graphs were drawn using Spectragryph 1.2.

Statistical Analysis

The yield data of gum production, its viscosity, and dried biomass of control (*X. campestris*) and five medicinal herbs were obtained from 5 replicates. Results obtained were averaged and the standard deviation of all the averaged (mean) results has been evaluated. Further, the multivariate approach of Principal Component Analysis (PCA) was used to identify which concentrations and combinations of medicinal herbs are effectively producing xanthan gum. The analysis was performed using PAST (4.0) software.

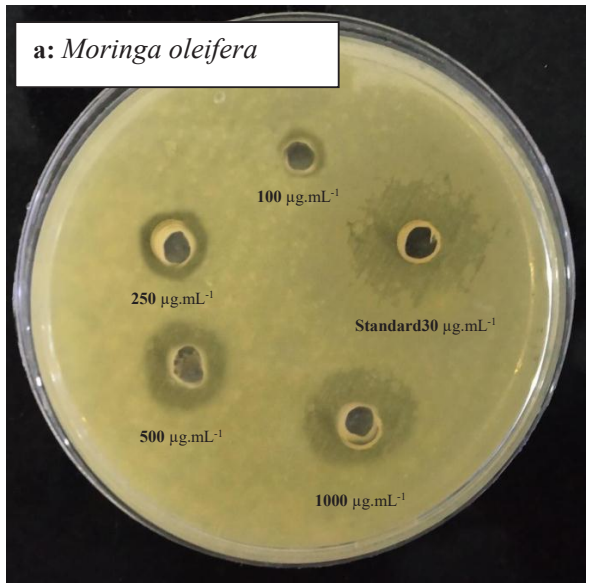
Table 2: Inhibitory effect of varying concentrations of different medicinal herbs against *Xanthomonas Campestris* in the form of zone of inhibition (ZOI).

Name of the Sample	Concentration ($\mu g.mL^{-1}$)	Diameter of ZOI (mm)
M. oleifera	100	12
	250	14
	500	19
	1000	23
	Kanamycin– 30 $\mu g.mL^{-1}$	27
B. monnieri	100	11
	250	13
	500	16
	1000	21
	Kanamycin– 30 $\mu g.mL^{-1}$	29
G. glabra	100	11
	250	14
	500	17
	1000	19
	Kanamycin– 30 $\mu g.mL^{-1}$	28
W. somnifera	100	11
	250	13
	500	18
	1000	22
	Kanamycin– 30 $\mu g.mL^{-1}$	29
A. platensis	100	12
	250	15
	500	17
	1000	23
	Kanamycin– 30 $\mu g.mL^{-1}$	27

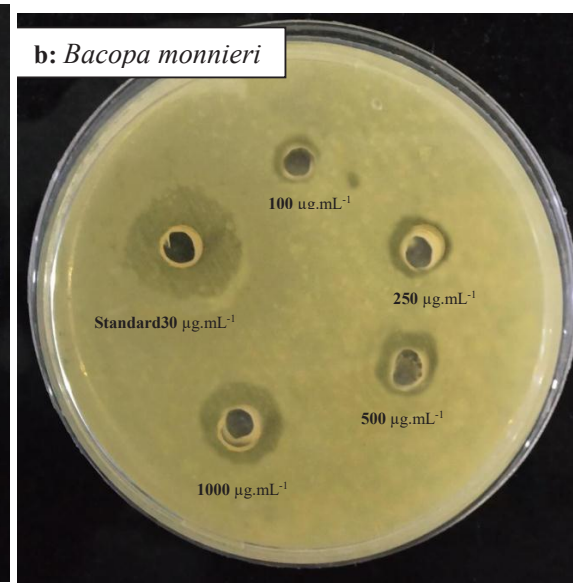
RESULTS

Antibiotic Susceptibility Test (AST)

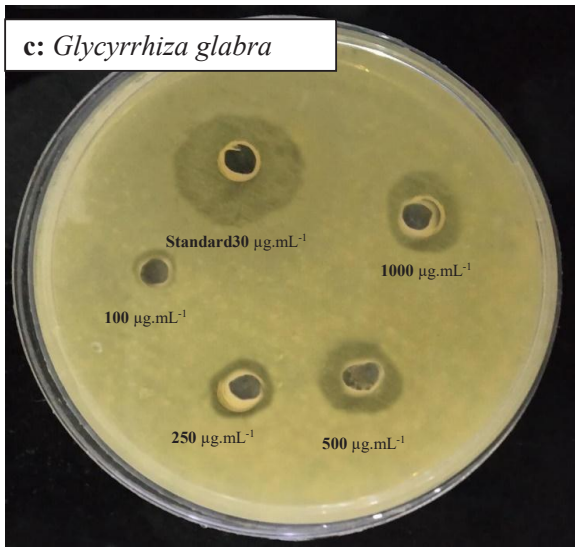
Antibiotic Susceptibility Test (AST) of five medicinal herbs was investigated for their effect on the growth of *X. campestris* by inhibition zone assay technique in-vitro conditions. In this study, post-incubation, the plates were observed for the zone of inhibition (ZOI) around the inoculated wells and the diameter of the zone is measured (Table 2). For this, growth is measured in different concentrations of methanol extract viz. 100 µg.mL^{-1}, 250 µg.mL^{-1}, 500 µg.mL^{-1} and 1000 µg.mL^{-1} and Kanamycin (30 µg.mL^{-1}) as a control (Table 2). *Moringa oleifera* produced ZOI of 23 mm, 19 mm, 14 mm, and 12 mm at concentrations of 1000 µg.mL^{-1}, 500 µg.mL^{-1},



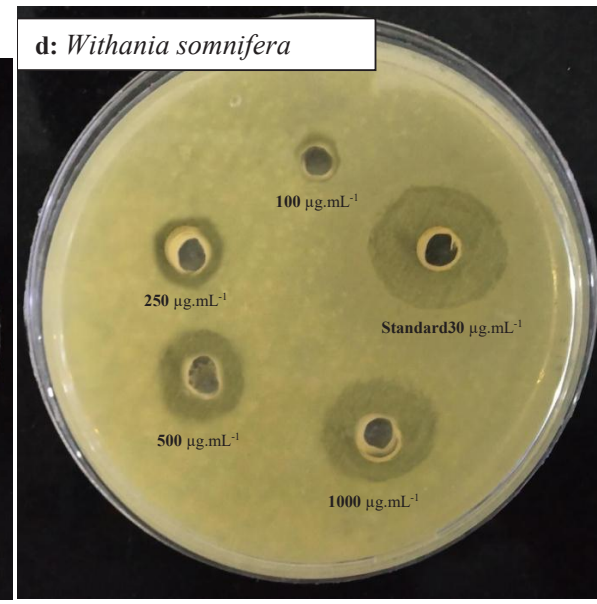
1(a)



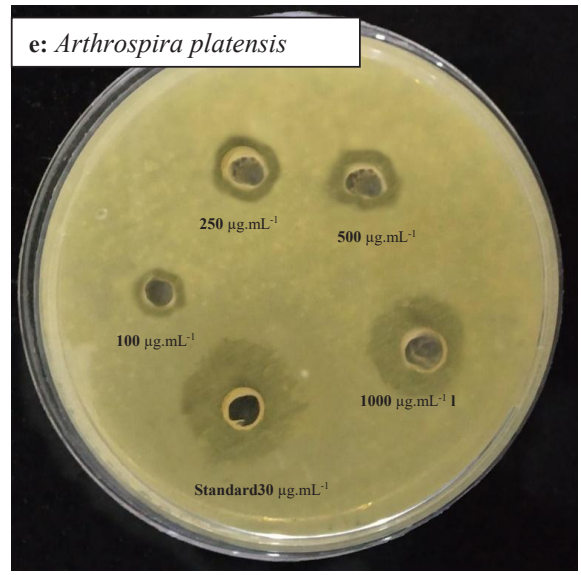
1(b)



1(c)



1(d)



1(e)

Fig 1. Antibacterial Study Test (AST) results of different herbs used in the study at dilution of 30 µg.mL^{-1} (standard), 100 µg.mL^{-1}, 250 µg.mL^{-1}, 500 µg.mL^{-1} and 1000 µg.mL^{-1} dilutions (a) *Moringa oleifera (b) Bacopa monnieri (c) Glycyrrhiza glabra (d) Withania somnifera (e) Arthrospira platensis.*

250 µg.mL^{-1}, 100 µg.mL^{-1}respectively and 27mm in control as shown in Fig. 1a. However, *Bacopa monnieri* produced ZOI of 21mm, 16mm, 13mm, and 11mm at concentrations of 1000 µg.mL^{-1}, 500 µg/ml, 250 µg.mL^{-1}, 100 µg.mL^{-1} respectively, and 29 mm in control (Fig. 1b). *Glycyrrhiza glabra* exhibited ZOI of 28mm in control, 19 mm, 17 mm, 14 mm, and 11mm at concentrations of 1000 µg.mL^{-1}, 500 µg.mL^{-1}, 250 µg.mL^{-1}, and 100 µg.mL^{-1} respectively (Fig. 1c). *Withania somnifera* exhibited ZOI of 29 mm in control, 22 mm, 18 mm, 13 mm, and 11mm at concentrations of 1000 µg.mL^{-1}, 500 µg.mL^{-1}, 250 µg.mL^{-1}, 100 µg.mL^{-1} respectively (Fig. 1d). *Arthrospira platensis* produced ZOI of 23 mm, 17 mm, 15 mm and 12 mm at concentrations of 1000 µg.mL^{-1}, 500 µg.mL^{-1}, 250 µg.mL^{-1}, 100 µg.mL^{-1} respectively, and 27mm in control (Fig. 1e). Results indicate that the ZOI of five plant extracts dissolved in methanol increased with an increase in concentration. However, the largest ZOI is reported in control of every sample.-

Xanthan Gum Production

All the results were obtained in 5 replicates in preoptimized conditions of 48hours. However, the mean of these replicate readings was taken for the analysis of the results obtained. Experiments were conducted to observe the effect of five medicinal herbs on the production of xanthan gum, viscosity, and dried cell biomass per liter of the solution.

For this, five herbs namely *M. oleifera, B. monnieri, G. glabra, W. somnifera,* and *A. platensis* along with a control (without herb) were studied for 48hrs. Table 3 presents the results of the yield of xanthan gum (obtained by adding medicinal herbs as an additive) per liter of the solution. Among all the studied herbs, the maximum yield of xanthan gum was obtained by the stress of *A. platensis* (9.12 g.L^{-1}) followed by *M. oleifera (*8.66 g.L^{-1}). However, in control (without any herb) the xanthan yield was determined as 7.41 g.L^{-1}. The results show that the presence of the medicinal herbs used in the study enhanced the production of xanthan gum in *X. campestris*. The highest yield of xanthan is observed by the stress of *A. platensis* followed by *M. oleifera* after 48 h. A present study is a novel approach, in which we studied the effect of medicinal herbs on xanthan production when used against *X. campestris* as antibacterial agents. Our results demonstrated the significantly enhanced production of xanthan gum under the influence of these herbs.

In the present study, the highest viscosity of xanthan gum was observed by the stress of *A. platensis* (905 mPa.s) followed by *M. oleifera* (717.2mPa.s), *G. glabra*, 675.2 mPa.s, *B. monnieri (*440.2 mPa.s) and *the* least by *W. somnifera* (485.2 mPa.s). However, in control, in which no herb was added, the viscosity of xanthan gum was determined as 430.20 mPa.s (Table 4).

Table 3: Effect of medicinal herbs on production of xanthan ($g.L^{-1}$).

S. No.	*M. oleifera*	*B. monnieri*	*G. glabra*	*W. somnifera*	*A. platensis*	Control
	8.74	7.52	8.12	7.84	9.00	7.41
	8.62	7.56	8.18	7.76	9.20	7.37
	8.74	7.57	8.20	7.69	9.18	7.47
	8.63	7.60	8.16	7.81	9.14	7.34
	8.59	7.51	8.22	7.78	9.09	7.45
Mean	8.66±0.07	7.55±0.04	8.18±0.04	7.78±0.06	9.12±0.08	7.41±0.05

In the present study, dried biomass of *A. platensis* (4 $g.L^{-1}$) was the maximum per liter of the solution as compared to other medicinal herbs used in the study. However, the dried biomass of control was observed as 3.18 $g.L^{-1}$, which was found least in amount as compared to samples having herbs. However, dried biomass in samples of *M. oleifera, B.monnieri, G. glabra,* and *W. somnifera* was determined as 3.82 $g.L^{-1}$, 3.42 $g.L^{-1}$, 3.76 $g.L^{-1}$, and 3.52 $g.L^{-1}$ respectively as shown in Table 5.

Spectroscopic Analysis using Fourier Transform Infrared (FTIR)

Fig. 2(a-e) represents the samples of xanthan gum from the medicinal herbs and Fig. 2f represents the control used in the study. By comparing the results, similar peaks (peaks in the range of 1550 – 1600 and 2700cm^{-1}) were found to be present in all the samples (Fig. 2a-e) as observed in control confirming the formation of xanthan gum. By comparing the intensity of the peaks, it is observed that some carbonyl double bonds, which can be from ketones, aldehydes, esters, or carboxyls may be present. A stretch in the range of 2700 cm^{-1} to 2835 cm^{-1} in control (Fig. 2f), *M.oleifera* (Fig. 2a), *B. monnieri* (Fig. 2b), *G. glabra* (Fig. 1c), *W. somnifera* (Fig. 2d) and *A. platensis* (Fig. 2e) attributes to $–CH_2$, RC-OH groups. A sharp peak at 2430 cm^{-1} is observed in Fig. 2d of *W. somnifera* indicating the P-H phosphine group. The absence of some of the peaks between the standard xanthan and plant additives explains the major structural changes caused by plants due to the influence of their bio-reducing phytochemical groups.

Principal Component Analysis (PCA)

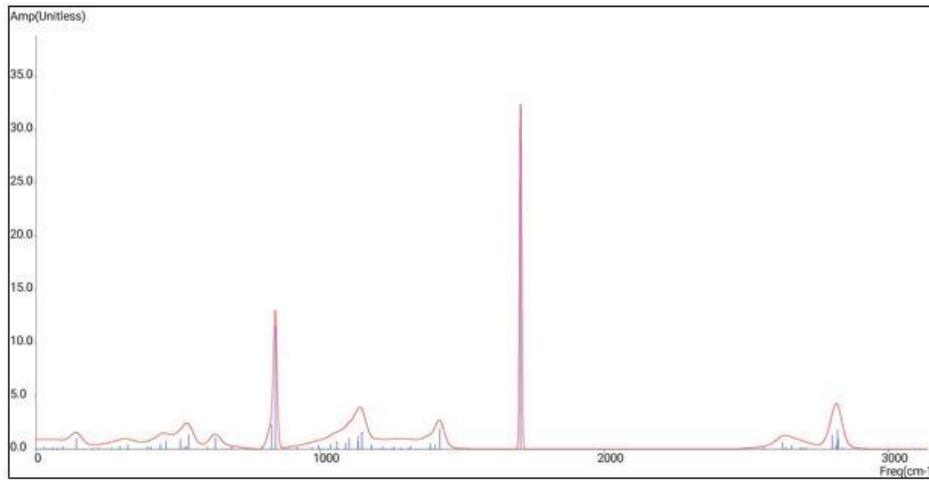
PCA analysis of the data matrix used 5 samples each of 5 herbs and control, which resulted in most of the data variance being explained by the first two principal components i.e., PC1 and PC2 (Fig. 3a-c). In Fig. 3a the first PC1 accounted for 43.73% of the total variance and the second PC 2 (name)

Table 4: Viscosity of xanthan gum produced under the stress of medicinal herbs (Pa.s).

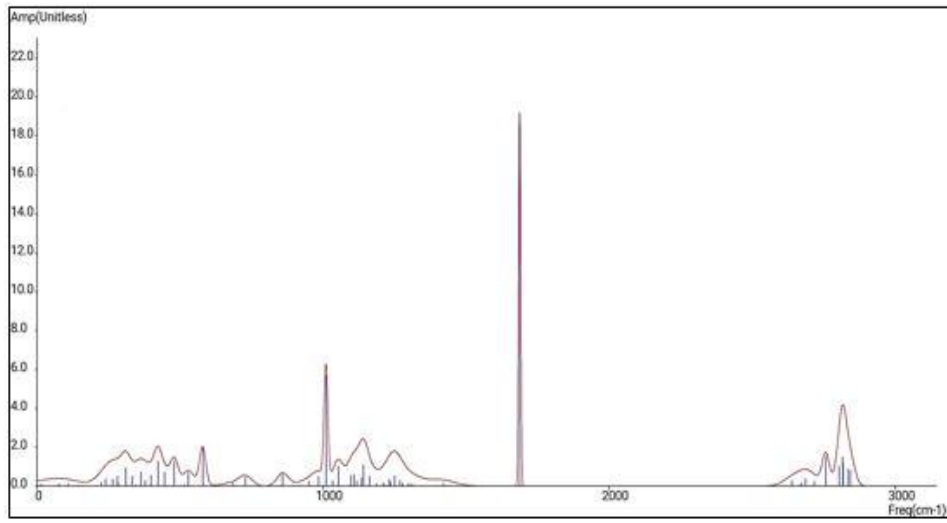
S.No.	*M. oleifera*	*B. monnieri*	*G. glabra*	*W. somnifera*	*A. platensis*	Control
	720	430	670	490	900	430
	714	444	675	484	910	427
	722	448	678	480	907	436
	718	452	673	487	905	424
	712	427	680	485	903	434
Mean	717±4.15	440±11.10	675±3.96	485±3.70	905±3.81	430±4.92

Table 5: Dried biomass produced after formation of xanthan by medicinal herbs ($g.L^{-1}$).

S.No.	*M. oleifera*	*B. monnieri*	*G. glabra*	*W. somnifera*	*A. platensis*	Control
	3.90	3.30	3.70	3.60	4.10	3.10
	3.70	3.50	3.80	3.50	3.90	3.10
	4.00	3.50	3.80	3.50	3.90	3.30
	3.80	3.60	3.70	3.60	4.10	3.20
	3.70	3.20	3.80	3.40	4.00	3.20
Mean	3.82±0.13	3.42±0.16	3.76±0.05	3.52±0.08	4.00±0.10	3.18±0.08



2(a)



2(b)

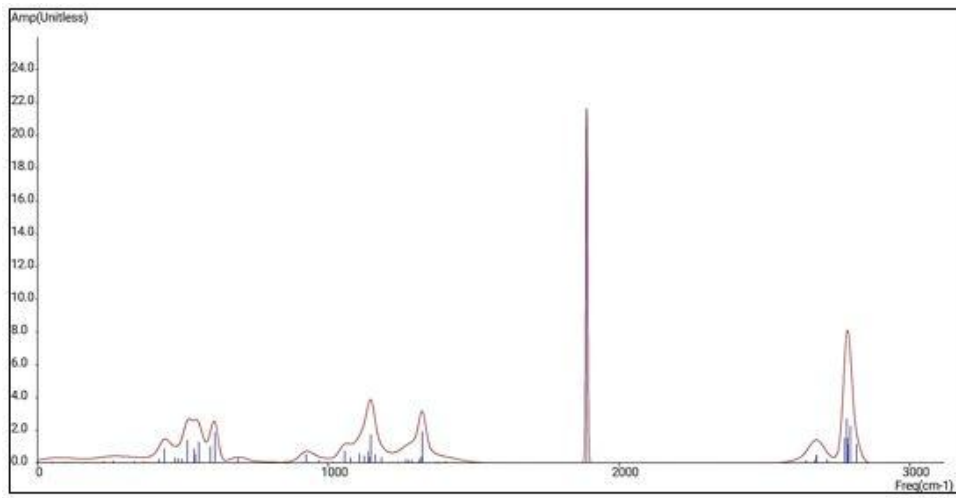
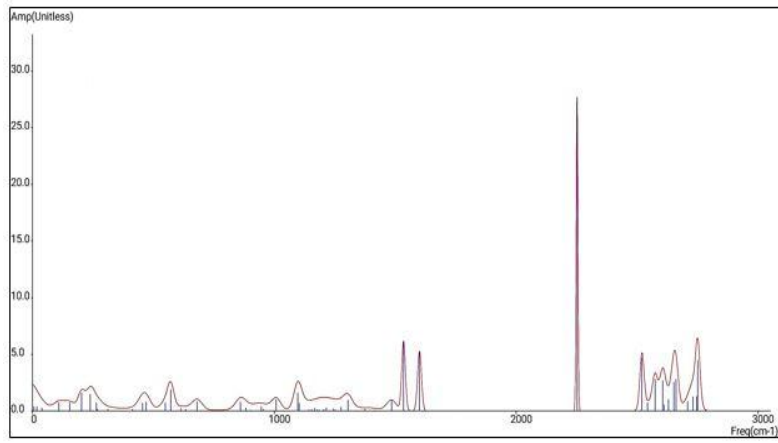
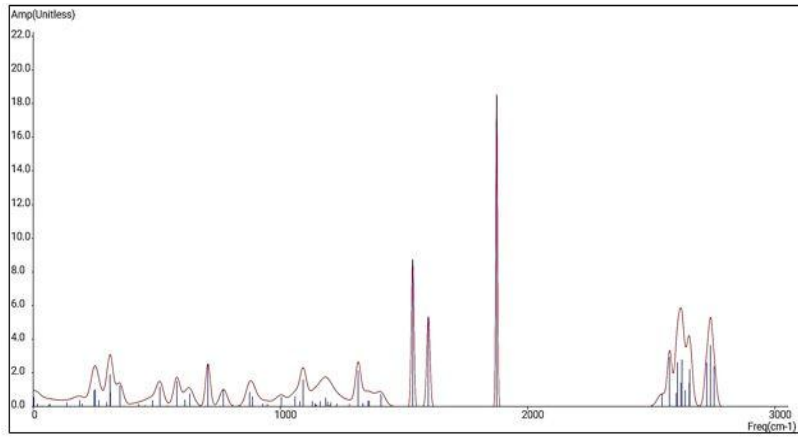


Fig. 2 cont....

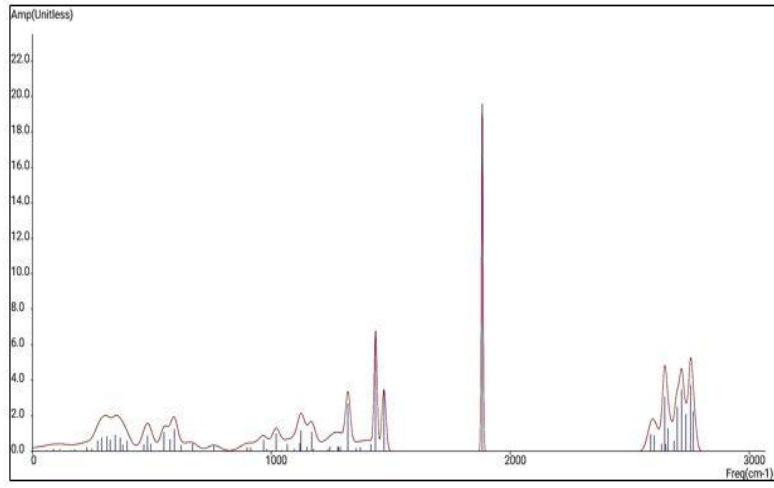
2(c)



2(d)



2(e)

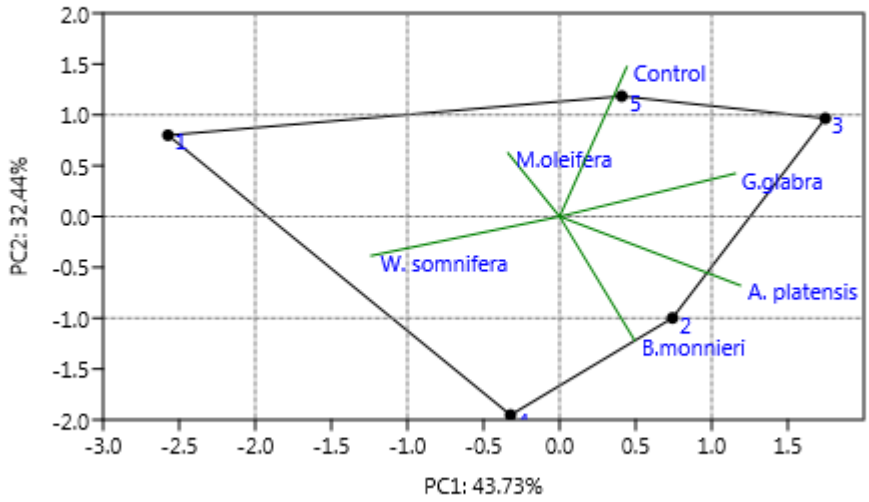


2(f)

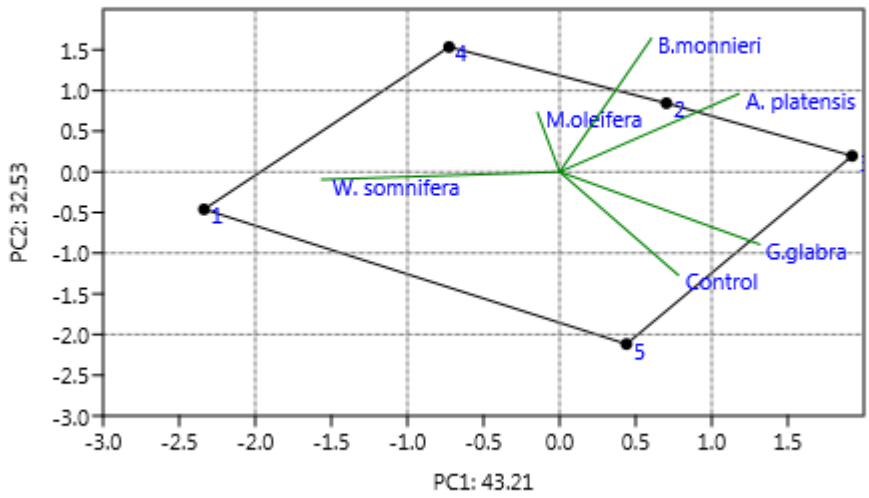
Fig. 2: FTIR spectra of xanthan gum produced with (a) *Moringa oleifera* (b) *Bacopa monnieri* (c) *Glycyrrhiza glabra* (d) *Withania somnifera* (e) *Arthrospira platensis* (f) Control (without any plant additive).

for 32.44%. As evident in Fig. 3a, *A. platensis* is the most important contributor to PC1 and Control is the most important contributor to PC2. *A. platensis* and *B. monnieri* are highly correlated whereas *W. somnifera* is almost unrelated to the rest of the two herbs in PC1. However, in PC2, all three variables are at almost equal angles, showing lesser correlation within them. *G. glabra* and *W. somnifera* fall in different clusters and show a negative correlation. Similarly, *M. oleifera* and *B. monnieri* are negatively correlated. *G. glabra* and *B. monnieri* are almost equally correlated to *A. platensis* as inferred from the vector angles of approx. 35°, whereas the vector angle of nearly 90° between the *A platensis* and Control showed a very poor correlation in the responses.

Fig. 3b, explains the graphical representation of the effect of different herbs on the viscosity of xanthan gum production. Here also, PC1 (43.21%) and PC2 (32.53%) show most of the variability. Control, *G. glabra,* and *W. somnifera* fall under PC1. Control and *G. glabra* are highly correlated to each other. However, *A. platensis, B. monnieri* and *M. oleifera* are equally correlated to each other and fall under PC2. Here, *B. monnieri* was found to contribute the most. Dried biomass obtained is presented in Fig. 3c with PC1 (47.04%) and PC2 (29.45%) using PCA. In PC1, *A. platensis* is found to be the only contributor, the rest of all herbs including control falls under PC2. *B. monnieri* and *M. oleifera* are found to be highly correlated.

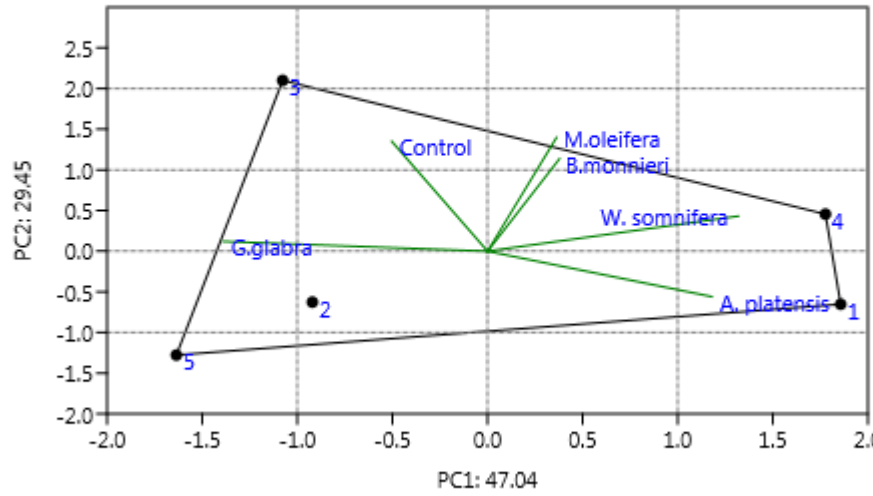


3(a)



3(b)

Fig. 3 cont....



3(c)

Fig. 3: Principal Component Analysis plot for (a) xanthan yield (b) viscosity of xanthan yield (c) dried biomass (only the first principal component, PC1, and the second principal component, PC2 are shown).

DISCUSSION

Sharma & Mehta (2001) tested leaf extracts from *Prosopis juliflora*, *Allium sativum, Vitis quadranquralis*, *Curcuma longa, Occimum sanctum,* and *Eucalyptis citridosa* for their antibacterial potential against *X. campestris*. Sheikh et al. (Sheikh et al. 2012) also studied the antimicrobial potential of eleven aqueous leaf extracts on *Xanthomonas campestris*, *Agrobacterium rhizogenes* and *Aspergillus fumigatus* by the formation of a zone of inhibition (ZOI). Pandey et al. (2011) also reported the antibacterial activity of *R. graveolens* in alcohol and water extracts against many Gram-negative bacterial and plant pathogenic fungi.

Various authors reported the enhanced xanthan yield by a change in their substrate, source of carbon, pH and agitation rates, etc. Recent studies conducted by Mudoi et al. (2013) and Renata et al. (2018) compared the waste residual molasses and carbon sources like glucose, sucrose, lactose, galactose, and maltose for the xanthan production. Lopez et al. (2001) reported the use of agro-industrial wastes such as olive mill wastewaters and date juice by-products (Salah et al. 2010) as a carbon source for xanthan production. In another study, Mohan & Babitha (2010) studied the effect of time on xanthan production and reported the maximum xanthan production after 48 hours. Chavan & Baig (2016) reported maximum xanthan production at 96hrs, after that it started decreasing. A study demonstrated that gum production tends to increase with high pH conditions (De Mello et al. 2016). However, Rana & Raval (2019) demonstrated the effect of carbon sources and incubation states on the viscosity of xanthan yield. Roncevic et al. (2019) observed the effect of different carbon sources (glucose, lactose, and starch) on the viscosity of xanthan yield and found the high quality of xanthan in terms of viscosity with glucose as compared to lactose and starch. In recent studies exopolysaccharides excreted by *A. platensis* under stress, conditions are determined and analyzed by Nagananthini et al. (2020). In the present study, the Elucidation of results shows that the addition of medicinal herbs not only exhibits antibacterial activity against *X. campestris* but also increases the yield of xanthan gum. These herbs are capable of creating stress when added during the stationary phase i.e. during 48 hrs. of incubation of culture in molasses medium, resulting in enhanced xanthan gum production. *A. platensis*, which is commonly known as *Spirulina* (filamentous, a gram-negative bacteria) was found most efficient, when used as an antibacterial drug against *X. campestris* and resulted in the highest yield of xanthan gum with maximum viscosity. Although, there could be many factors affecting the process which need to be further studied for a better understanding of the metabolism taking place.

CONCLUSION

The present investigation established novel research on the dual benefits of five medicinal herbs against the *X.campestris.* The antibacterial nature of five medicinal plants not only inhibits the growth of *X.campestris* but also increases the production of xanthan gum (which is used commercially in the food and pharmaceutical industries mainly). Characterization using FTIR spectroscopy confirms the formation of xanthan gum by the five plants when compared with

the control. Results illustrated that *Moringa oleifera and Arthrospira platensis* are the most promising herbs for the production of xanthan along with their antibacterial potentials against *X. campestris*. In the future, this opens the prospects of these medicinal herbs exclusively against *X. campestris* as an eco-friendly and safe production of xanthan gum.

ACKNOWLEDGEMENT

Great thanks to the Faculty of Microbiology, Amity Institute of Microbial Technology, Amity University, Noida for their continuous guidance and support. I would also like to thank Mr. Prasanth, Simbioen Labs, and Scientific Services for his support. Thanks to Dr. Anjana Kamra for her contribution to drafting. Special thanks to Mr. Girish Parikh (Ex- Managing Director, Zandu Pharmaceutical Works Ltd) for his inspiration.

REFERENCES

Abedinzadeh, S., Torbati, M. and Azadmard-Da-mirchi, S. 2016. Some qualitative and rheological properties of virgin olive oil-apple vinegar salad dressing stabilized with xanthan gum. Adv. Pharma. Bull., 6(4): 597-606. https://doi.org/10.15171/apb.2016.074

Bajic, B., Dodic, J. and Roncevic, Z. 2014. Biosynthesis of xanthan gum on wastewater from the confectionery industry. Analecta, 8(2): 13-17.3. https://doi.org/10.14232/analecta.2014.2.13-17

Bajic, B., Roncevic, Z. and Puskas, V. 2015. White wine production effluents are used for the biotechnological production of xanthan. J. Process. Energy Agric., 19(1): 52-55.

Chavan, S. and Baig, M.M.V. 2016. Relationship of biomass and xanthan gum production by *Xanthomonas campestris*: optimization of parameters. Brit. Biotechnol. J., 11(1): 1-8. https//doi.org/10.9734/BBJ/2016/22431

Clarke, M.A. 2003. Syrups Encyclopedia of Food Sciences and Nutrition. Second edition. MacGraw Hill, USA

De Mello, L.M., Borges, C.D. and de Oliveira, T.D. 2016. Structure of xanthan gum and cell ultrastructure at different times of alkali stress. Brz. J. Microbiol., 47: 102-109. https://doi.org/10.1016/j.bjm.2015.11.006

Lopes, B., De Monaco, M., Lessa, V.L. and Silva, B.M. 2015. Xanthan gum: properties, production conditions, quality, and economic perspective. J. Food Nutri. Res., 54(7): 185-194.

Dogan, M., Kayacier, A. and Erhan, I.C. 2007. Rheological characteristics of some food hydrocolloids processed with gamma irradiation. Food Hydrocoll., 21: 392-396. https://doi.org/10.1016/j.foodhyd.2006.04.010

Dos Santos, F.P., Antonio, M. and Oliveira, J. 2016. Bioconversion of agro-industrial wastes into xanthan gum. Chem. Eng. Transac., 49: 145-150. https://doi.org/10.3303/CET1649025

Garcia-Ochoa, F., Santos, V.E., Casas, J.A. and Gómez, E. 2000. Xanthan gum: Production, recovery, and properties. Biotechnol. Adv., 18: 549-579. https://doi.org/10.1016/S0734-9750(00)00050-1

Infee Sherley, K. and Priyadharshini, R.D. 2015. Review on the production of Xanthan gum in batch and continuous reactors. Int. J. Chem. Tech. Res. 8(2): 711-717.

Kawahara, H. and Obata, H. 1998. Production of xanthan gum and ice-nucleating material from whey by *Xanthomonas campestris* pv.translucens. Appl. Microb. Biotech., 49: 353-358. https://doi.org/10.1007/s002530051181

Kennedy, J.F. and Bradshaw, I.J. 1984. Production, properties and applications of xanthan. Progress Ind. Microbiol., 19: 319-371.

Lopez, M.J., Moreno, J. and Ramos-Cormenzana, A. 2001 *Xanthomonas campestris* strain selection for xanthan production from olive mill wastewaters. Water Res., 35: 1828-1830. https://doi.org/10.1016/S0043-1354(00)00430-9

Magaldi, S., Mata-Essayag, S., De Capriles, C.H., Perez, C., Colella, M.T., Olaizola, C. and Ontiveros, Y. 2004. Well diffusion for antifungal susceptibility testing. Int. J. Infect. Dis., 8(1): 39-45.

Mohan, T.S. and Babitha, R. 2010. Influence of nutritional factors on xanthan production by Xanthomonas *malvacearum*. Arch. App. Sci. Res., 2(6): 28-36.

Mudoi, P., Bharali, P. and Konwar, B.K. 2013. Study on the effect of ph, temperature, and aeration on cellular growth and xanthan production by Xanthomonas *campestris* using waste residual molasses. J. Bioprocess. Biotechnol., 3(2): 1000135.

Nagananthini, G., Rajapriya, S. and Arivuvel, P.S. 2020. Extraction and optimization of extracellular polysaccharide production in S*pirulina platensis* MK 343101. Int. J. Sci. Technol. Res., 9(4): 1-5.

Ng, H.S., Kee, P.E. and Yim, H.S. 2020. Recent advances in the sustainable approaches for conversion and reutilization of food wastes valuable bioproducts. Bioresour. Technol., 302: 12289. https://doi.org/10.1016/j.biortech.2020.122889

Pandey, P., Mehta, A. and Hajra, S. 2011. Evaluation of the antimicrobial activity of *Rutagraveolens* stem extracts by disc diffusion method. J. Phytol., 3(3): 92-95.

Petri, D.F.S. 2015. Xanthan gum: A versatile biopolymer for biomedical and technological applications. J. App. Polym. Sci., 132: 1-13. https://doi.org/10.1002/app.42035

Rana, B.M. and Raval, A.A. 2019. Isolation, production, and characterization of the polysaccharide "xanthan gum" from *Xanthomonas* sp. Int. J. Curr. Microbiol. App. Sci., 8(5): 1019-1030.

Renata, A.T., Adriel, P.M. and Carlos, B. 2018. Impact of a carbon source and stress conditions on some properties of xanthan gum produced by *Xanthomonas campestris* pv. *Mangiferae indicae*. Biocataly. Agric. Biotech., 15: 167-172. https://doi.org/10.1016/j.bcab.2018.06.003

Roncevic, Z.Z., Zahovic, I.E. and Pajcin, I.S. 2019. Effect of carbon sources on xanthan production by *Xanthomonas* spp. Isolated from pepper leaves. Food Feed Res., 46(1): 11-21. https://doi.org/10.5937/FFR1901011R

Salah, R.B., Chaari, K. and Besbes, S. 2010. Optimization of xanthan gum production by palm date (*Phoenix dactylifera L.*) juice by-products using response surface methodology. Food Chem., 121: 627-633.

Sarbatly, R. and England, R. 2006. Review of xanthan gum production from unmodified starches by *Xanthomonas compressors* sp. Enzyme Microbial. Tech., 39(2): 197-207. https://doi.org/10.1016/j.foodchem.2009.12.077

Sharma, P. and Mehta, B.P. 2001. Antibacterial activity of plant extracts to phytopathogenic *Xanthomonas campestris pv. Campestris* causing black rot of cabbage. J. Mycol. Pathol., 31: 111–112.

Sheikh, M., Malik, A., Meghavanshi, M. and Mahmood, I. 2012. Studies on some plant extracts for their antimicrobial potential against certain pathogenic micro-organisms. Am. J. Plant Sci., 3(2): 209-213. https://doi.org/10.4236/ajps.2012.32025

Souw, P. and Demain, A.L. 1979. Nutritional studies on xanthan production by *Xanthomonas campestris* NRRL-B-1459. Appl. Environ. Microbiol., 37: 118–119. https://doi.org/10.1128/aem.37.6.1186-1192.1979

Stredansky, M. and Conti, E. 1999. Xanthan production by solid-state fermentation. Process. Biochem., 34: 581-587. https://doi.org/10.1016/S0032-9592(98)00131-9

Suput, D., Lazic, V., Popovic, S. and Hromis, N. 2015. Edible films and coatings –sources, properties, and application. Food Feed Res., 42(1): 11-22. https://doi.org/10.5937/FFR1501011S