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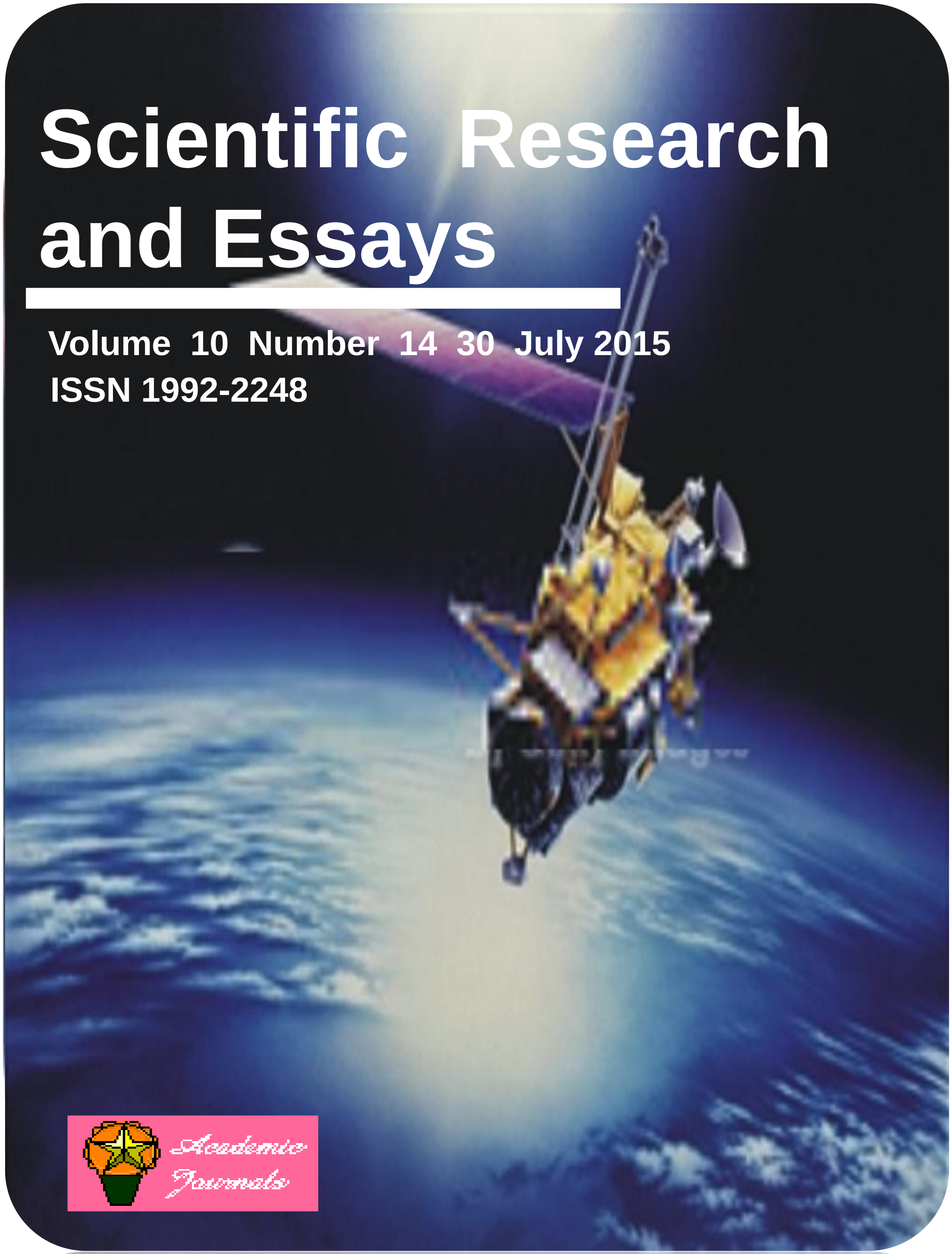

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Scientific Research and Essays

Full Length Research Paper

Structural damping identification using a recursive Kalman filter

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Much research has been done in developing techniques for identifying the structural damping of physical systems. Such techniques are always accompanied by the development of an analytical model of the ideal system and its comparison with experimental data obtained in laboratory. Also, flexible systems are difficult to be modeled, but the authors can use an approximation, supposing that a flexible system, composed by a cantilever beam, can be similar to a massa-spring-damper system. In this work, are shown a recursive technique for identifying the structural damping of a physical system and its applications. The authors identified the structural damping of a system consisting of a flexible beam clamped where the authors use a mass-spring-damper model to represent it. The excitation of the system was carried out using an impact hammer in order to use such data at the input of the analytical model obtained for the system. For the flexible system, the authors implemented the methodology of recursive Kalman's filter, in order to identify the flexibility and damping coefficients. The results show that the technique has been successfully applied once the error obtained by comparing the experimental and analytical data is quite small.

Key words: Identification, modeling, structural damp, Kalman filter.

INTRODUCTION

The area of identification of damping of physical systems still has several questions to be answered by the scientific community. The effects of damping are well known, but its precise characterization is still an unsolved problem. In Pilkey et al. (1997), the authors propose two methods for identification of damping, an interactive and another that uses the method of least squares. In Adhikari (2002), the author proposes a method, based on the poles and waste measurements of transfer functions associated with the method of Lancaster. In Pradhan and Modak (2012), the authors address the issue of identification of damping matrix of a structure by posing it

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as a finite element damping matrix updating problem. According to the authors, the formulation developed seeks to separate updating of the damping matrix from that of updating of the stiffness and the mass matrix, reducing the difference between the complex FRFs. In Andrianne and Dimitriadis (2012), the authors show a new technique to identify the damping of linear systems. The ability of such technique to estimate the mode shapes and the modal damping is demonstrated, by the authors, on a simulated mass–spring–damper system. In Arora (2014), the author proposes and tests a new structural damping identification method using normal frequency response functions which are obtained experimentally. According to the author, the test has the objective that the damped finite element model is able to predict the measured FRFs accurately.

In Pan and Wang (2014), the authors outline a complex mode procedure for identifying the exponential damping model and discuss its applicability and limitations. Also a new iterative method for relaxation factor is proposed, without using the full set of modal data. The authors also state that the finite element model updated method for the systems with exponential damping can predict accurately not only the natural frequencies but also the FRFs of the systems. In Arora (2015), the author proposes a method, which is a FRF-based method and overcomes the problem of closely spaced modes for damping identification. The author also states that the accuracy of identified structural damping matrix depends upon the accuracy of finite element model. To address this issue, in this paper was set up a simple experiment in the Vibration Laboratory, Department of Mechanical Engineering at University of Taubaté (UNITAU). The experiment used as input an impulse from an impact hammer, whose calibration is performed in the laboratory. The experiment consists of a flexible beam instrumented with an accelerometer on its free tip and also excited by the impact hammer.

This paper presents the analytical modeling of the physical system, together with studies to validate its models through experimental tests and studies of parametric identification of the parameters of the systems in the time domain. An impulse (applied by the impact hammer) excites the beam and the excitation of the tip of the beam, measured by an accelerometer, is acquired for further analysis. In order to do the system's parameters identification, the authors implemented a recursive Kalman filter, using the Octave software. Preliminary results show a good agreement between experimental and analytical models, leading us to conclude that the computational procedure works quite satisfactory.

CALIBRATION OF THE IMPACT HAMMER

Aiming to increase the reliability of the experimental data, the authors chose to calibrate the impact hammer in the laboratory, setting up an experimental set-up consisting of a known mass and a capacitive accelerometer (model MMA1220D Micro machined Motorola), previously calibrated (the accelerometer sensibility was supplied by the manufacturer), as illustrated in Figure 1.

This experiment is suggested by the equipment manufacturer (PCB Piezotronics) and involves the application of Newton's 2nd law to the result of applying an impulse to a known mass:

$$F = ma \tag{1}$$

Writing the equations in terms of the sensibility of the accelerometer and impact hammer, the authors have:

$$\frac{V_F(t)}{S_F} = m\frac{V_a(t)}{S_a} \Longrightarrow S_F = \frac{S_a}{m}\frac{V_F(t)}{V_a(t)} \tag{2}$$

In Equations (1) and (2), *a* is the acceleration of gravity. *F* is force, *m* is the mass used in the experiment (*m*=200.23g), V_F is the voltage peak value registered by the impact hammer, S_F is the sensitivity of the impact hammer, *Va* is the voltage peak registered by accelerometer and *Sa* is the sensitivity of the accelerometer, supplied by the manufacturer (*Sa*=250mV/g). Figures 2 and 3 illustrate the temporal response of the impact hammer and the accelerometer, after the application of an impulse to the mass.

Replacing numerical values in Equation (2), the authors obtain:

$$S_m = \frac{250\times 10^{-3}\times 9.81}{200.23{\times}10^{-3}}.\frac{(-0.245109)}{4.99642} = -0.060086\left[\frac{mV}{N}\right]$$

Once given the sensitivity of the impact hammer, the experiment was assembled as described in this research.

IDENTIFICATION OF A STRUCTURAL DAMPING OF A CANTILEVER BEAM

Approximate mathematical model of a cantilever beam

The simplified model of a simple cantilever beam, shown in Figure 4 can be obtained comparing it to a mass-spring-damper system. Damping constant *b* is due to structural damping of the beam, and flexibility is given by *k*. Writing kinetic, potential and dissipation energy of the system, the authors have:

$$T = \frac{1}{2}m\dot{x}^2 \tag{3}$$

$$V = \frac{1}{2}kx^2 \tag{4}$$

$$R = \frac{1}{2}b\dot{x}^2 \tag{5}$$

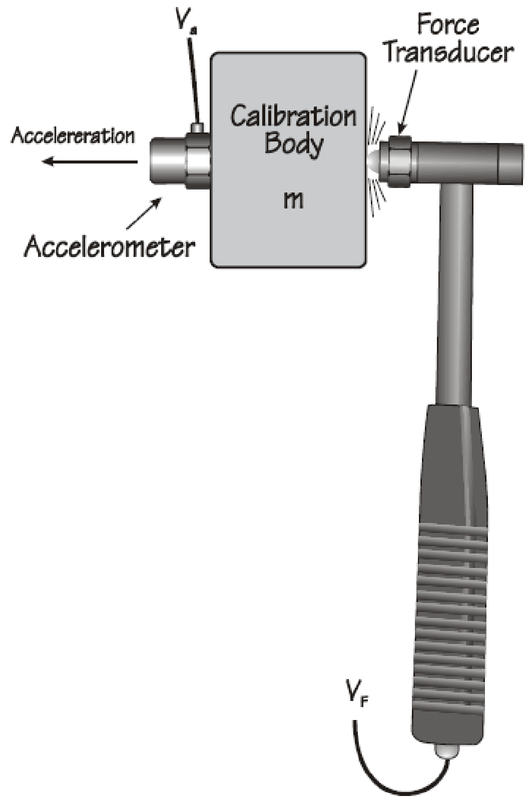

Figure 1. Scheme for the calibration of the impact hammer.

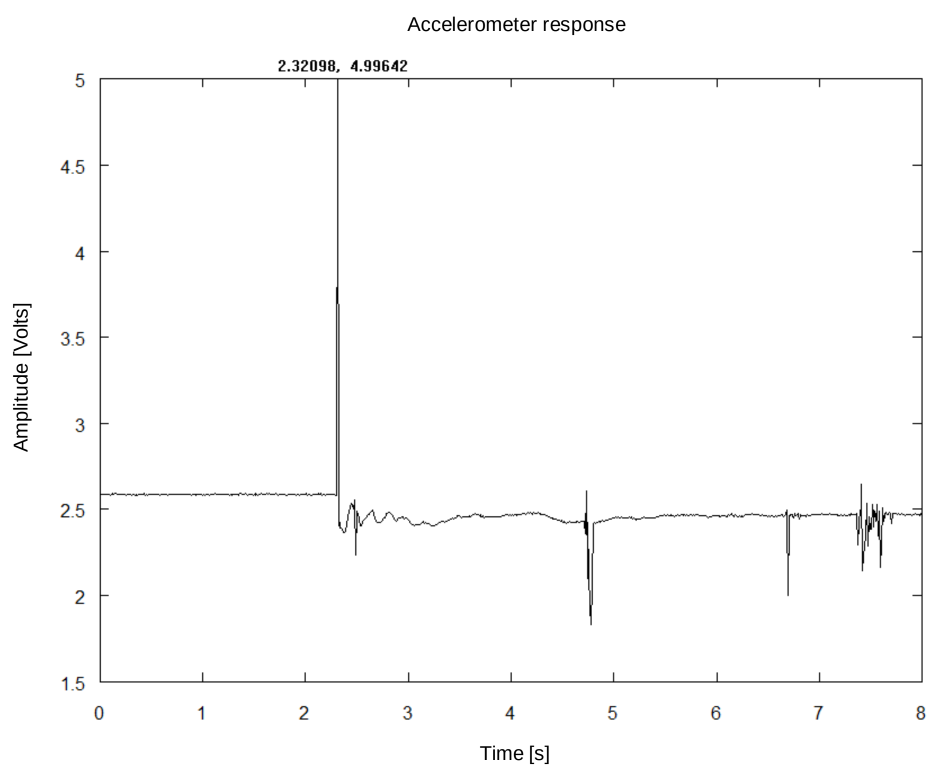

Figure 2. Output of the accelerometer after application of an impulse to the mass.

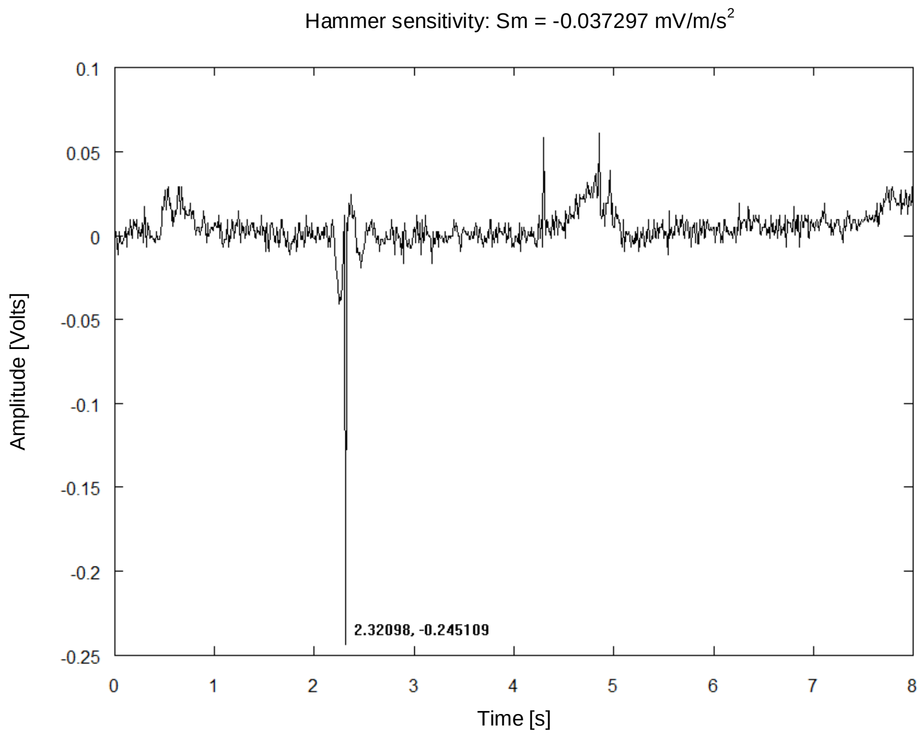

Figure 3. Output of impact hammer after applying an impulse to the mass.

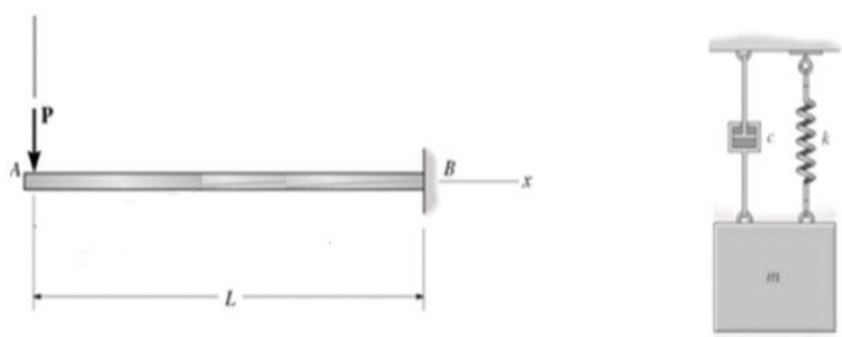

Figure 4. Schematic of the cantilever beam system and a mass-spring-damper system.

Using Lagrange's equations Wellstead (1979), one obtains the model described by the classical Equation (6).

$$m\ddot{x} + b\dot{x} + kx = f(t) \quad (6)$$

Where *f(t)* is an external force applied to the system to excite him. In the Laboratory of Mechanical Vibrations, an experiment was set up with a cantilever beam, with the characteristics described in Table 1.

The data acquisition equipment was composed by a capacitive accelerometer (model MMA1220D Micro machined Motorola), connected to a signal acquisition board (NI USB-6009 from National Instruments) connected to a USB port on a PC compatible. The experimental data acquisition program was developed using the graphical language LabView ®. The accelerometer was attached to the free tip of the beam and the system was excited with an impulse applied to the impact hammer (Figure 5).

Digital filtering and numerical integration

Accelerometers are sensors widely used in engineering applications. It can be helpful to perform modal analysis

Table 1. Physical parameters of the cantilever beam.

Aluminum density	ρ	2710	Kg/m^3
Aluminum Young's modulus	E	7.1E10	N/m^2
Beam's width	*eb*	31.7 E-3	m
Beam's height	*hb*	3.3E-3	m
Beam's length	L_b	0.7	m
Beam's cross-section area	A	10.46E-5	m^2
Beam's moment of inertia	I	9.493E-11	m^4

Figure 5. Experiment mounted with a cantilever beam.

and many others analysis in frequency domain. If the authors are not concerned with the signal amplitude, this analysis could be done without worries. In this case, the authors are interested to have a precisely measurement of a velocity and displacement at the position where the accelerometer was located. This will lead us to be sure that the signal amplitude is correct. Also, the authors need to perform a numerical integration of the signal twice. Figure 6 represents a scheme about what the authors implement at first.

The filter that the authors choose was a high pass Butterworth with five terms and cutoff frequency in 5Hz. Those values were chosen empirically. To reach at the results, the authors used the routine available in Octave® software, called *butter*, which designs the filter by calculating its coefficients and the routine *filter* which applies the calculated coefficients to the signal. The numerical integrator used, implements the Trapezoidal Rule and the authors used the routine also available in Octave® software called *trapz*. Figure 7 and 8 illustrate the results of the application of an impulse to the cantilevered beam.

For the identification of damping parameter (*b*) and flexibility parameter (*k*) of the cantilever beam, the authors used the technique of recursive Kalman filter (Bierman, 1977). For the assembly process, Equation (6) should be rewritten as:

$$m\ddot{x} - f(t) = -b\dot{x} - kx \tag{7}$$

In Equation (7), the values of $\ddot{x}$ and $f(t)$ are the output of the accelerometer and impact hammer respectively and the values $\dot{x}$ and x were obtained by integrating twice the signal from the accelerometer, like previously mentioned. The system was therefore placed in the form:

$$z = Aw + v \tag{8}$$

Comparing Equation (7) and (8) the authors can do the following assignments:

$$z = m\ddot{x} - f(t) \tag{9}$$

$$A = [\dot{x} \quad x] \tag{10}$$

Figure 6. Scheme to obtain the velocity and displacement of the free tip of the beam.

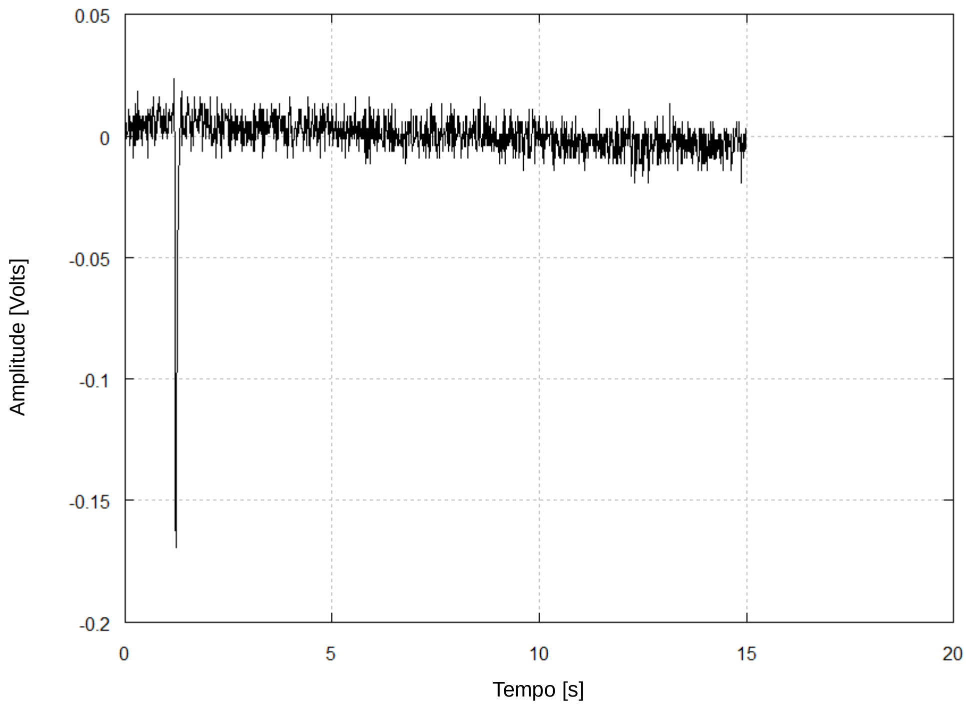

Figure 7. Impact Hammer output after applying an impulse to the cantilever beam.

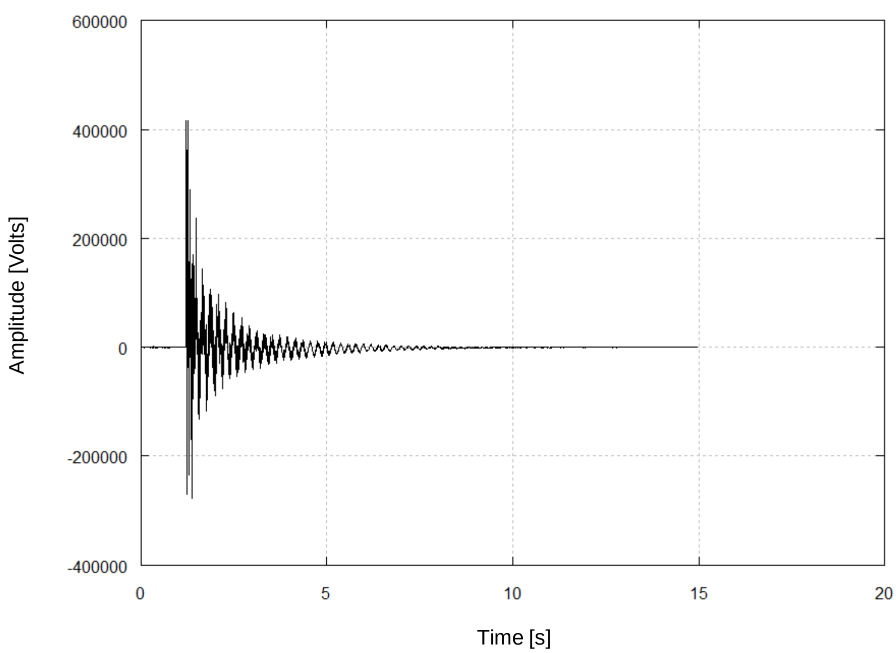

Figure 8. Acceleration of the free tip of the beam after application of the pulse.

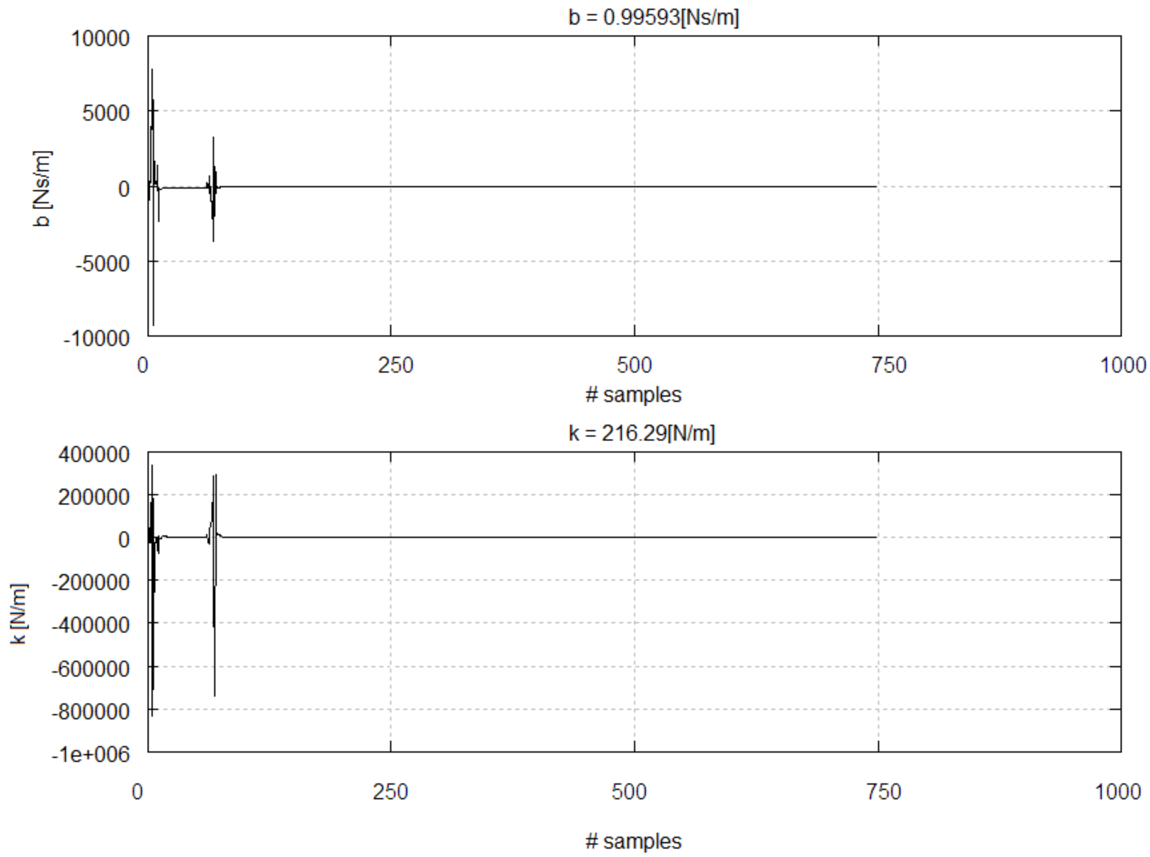

Figure 9. Results of the recursive Kalman filter in the calculation of *b* and *k*.

$$w = [-b \quad -k]^T \qquad (11)$$

And v is the error associated with the observations.

The recursive Kalman filter algorithm (Appendix A), implemented in Octave software version 3.0.0, has calculated the following values for *k* and *b*, respectively: 216.29$[N/m]$ and 0.99693$[Ns/m]$. The graph (Figure 9) shows the robustness of the method, which converge shortly after the sample number 200of 1000 samples considered.

With the parameters identified, the authors compare the analytical model, given by Equation (6) and the experimental model in the graphic illustrated in Figures 10 and 11.

Figure 11 also shows the comparison of the results but with a more favorable range, in order to highlight the agreement between the experimental and analytical results.

DISCUSSION

The identification of the damping in flexible systems is still a challenge to be overcome. The use of a recursive technique shows that it is possible to reach a reasonable estimation with a reliable method, the recursive Kalman filter. The technique is very simple to implement and show a very nice convergence using only a few samples.

CONCLUSIONS AND FURTHER WORK

This paper presents the identification of the damping and flexibility parameters of a physical system composed by a cantilever beam. To validate the identified parameters a representative experiment were assembled in laboratory. The experiment was monitored using the LabVIEW software and a data acquisition board (NI USB -6009 manufactured by National Instruments). The authors implemented the method of recursive Kalman filter to identify the damping parameters (b) and flexibility (k) of the system. Results obtained show (Figures 10 and 11) good agreement between the analytical model and experimental, suggesting success in the identification. The work is in progress and proposals for its continuation are: (a) work with an even simpler system with concentrated parameters, but with more degrees of freedom and (b) using the full model (distributed

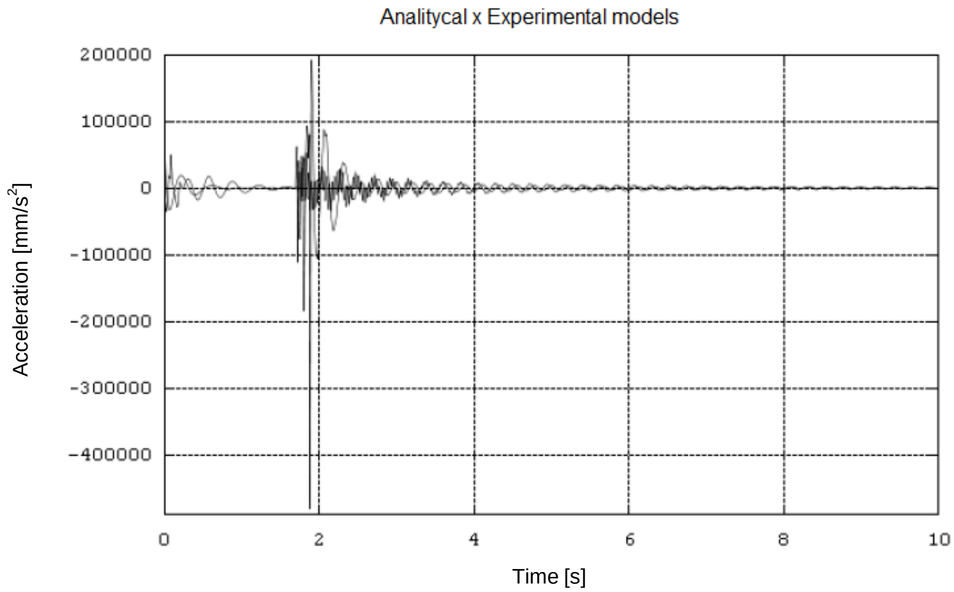

Figure 10. Comparison between analytical and experimental models.

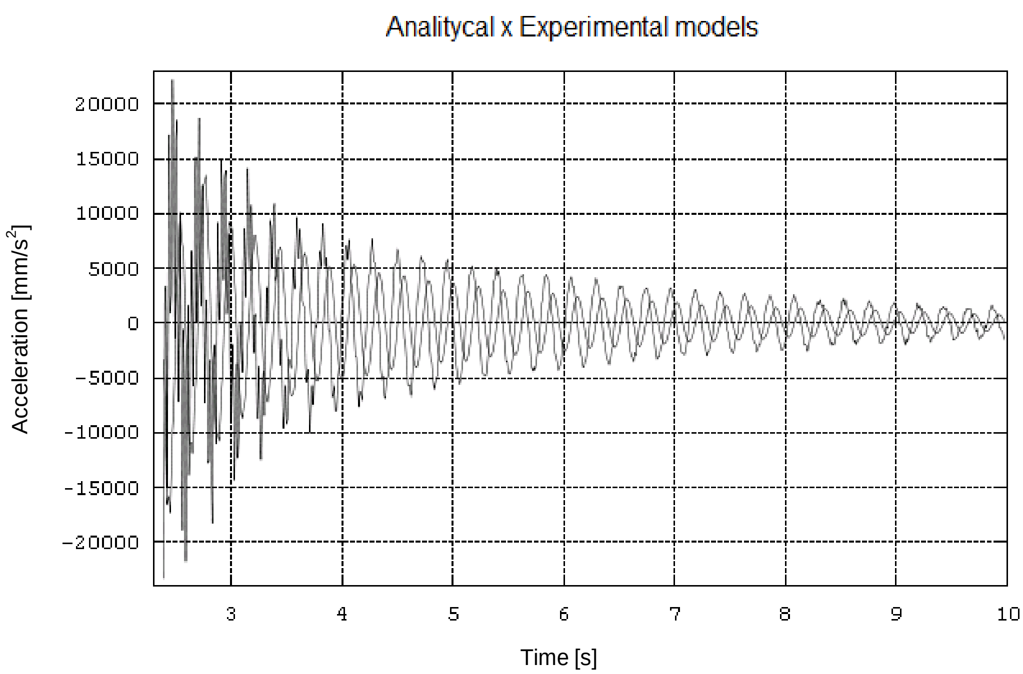

Figure 11. New comparison between the analytical and experimental models.

parameter) of the cantilever considering their flexibility and degrees of freedom and carrying out the identification of the structural damping matrix.

Conflict of Interest

The authors have not declared any conflict of interest.

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APPENDIX

A – Recursive Kalman filter routine

```
function [x,P] = kalman(x,A,z,N,P);

sigma = 1;
delta = z;
for i=1:N,
    v(i) = 0.0;
    for j=1:N, v(i) = v(i) + P(i,j)*A(j); end;
    delta = delta - A(i) * x(i);
    sigma = sigma + A(i) * v(i);
end;

%                         t                     t
%Comment : v = PA  , sigma = APA  + 1, and
%          delta = z - Ax have been computed
%
sigma = 1.0/sigma;
for i=1:N,
   K(i) = v(i) * sigma;  % Kalman gain
   x(i) = x(i) + K(i) * delta;
   for j=1:N, P(i,j) = P(i,j) - K(i)*v(j); end;
   P(j,i) = P(i,j);   % utilized simmetry
end;

% Comment : Kalman gain, updated estimate and
% optimal covariance have been computed
%

for i = 1:N,
   v(i) = 0.0;
   for j=1:N, v(i) = v(i)+P(i,j) * A(j); end;
end;

for j=1:N,
    for i=1:j,
       s = 0.5 * (P(i,j) - v(i) * K(j) + P(i,j) - v(j) * K(i));
       P(i,j) = s + K(i) * K(j);  % stabilized update
       P(j,i) = P(i,j);
     end;
end;
```

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Scientific Research and Essays

Full Length Research Paper

Impact analysis of front line demonstration of rice (*Oryza sativa*) on the yield, economics and farmer's knowledge in temperate region of India

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Impact analysis of Front Line Demonstrations (FLD's) of rice on yield, economic returns, level of knowledge and adoption extent was conducted through a study in temperate region of northern India, in which 240 participating farmer respondents and 240 non-participating farmer respondents from 5 representative villages in four districts were selected through stratified random sampling method for the purpose. Based on the data collected during 2014 and the interviews with the two categories of farmers, the study reveals considerable increase in the grain yield (27.75%), economic returns (27.41%) and knowledge (43.70%) among participant farmers as compared to non-participant farmers. Correlation reveals that among participating farmers age, literacy and extension contract were positively and significantly associated with the improved knowledge about rice production in all districts. In respect of non-participating farmers age, literacy, operational land holding, extension contact and farm diversification were contributing positively and significantly towards improved rice production in all districts. However, among the participating farmers exposure to different media was negatively associated with the improved knowledge in all districts. The results of regression analysis revealed that age, literacy, extension contact, attitude towards farm diversification variables among participating farmers have indeed helped in contributing to farmer's knowledge through FLDs.

Key words: Front Line Demonstration (FLD), yield gap analysis, economics, grain yields, knowledge.

INTRODUCTION

Rice is the staple food of over half of the world's population. It is the predominant dietary energy source for 34 countries in Asia, Pacific, North and South America and Africa. Rice provides 20% of the world's dietary energy supply. It is the most important food crop of the developing world and the staple food for more than 60% of the Indian population (Anomymous, 2012). It is one of the most important food crops of India in term of area, production and preferred food item throughout the country. India is the second largest producer and

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consumer of rice in the world, where production crossed the mark of 100 million MT in 2011-2012, which accounts for 22.81% of global production in that year. India needs to produce 120 million tons by 2030 to feed its one and a half billion plus population (Anonymous, 2013). The scenario needs cutting edge technologies for increasing rice production in India. Although productivity of rice has increased from 1984 kg per hectare in 2004-2005 to 2372 kg ha^{-1} in 2011-2012, due to development of high yielding varieties with site specific technology, but huge technological and extension gaps are constantly being reported, which tantamount to identify causes through indept research.

Front Line Demonstration (FLD) has been used as an useful extension tool to demonstrate HYV along with production, protection and management practices in the farmer's field under different agro-climatic regions and farming situations. The improved cultivation practices followed in the national demonstrations have already shown high yield potentials (Anonymous, 2012). But knowledge behaviour of general farmers towards these practices is not known and hardly any systematic research has done to explore these areas. Therefore, it is very essential to conduct investigation on Front Line Demonstrations on rice to assess their effectiveness and efficacy towards enhancement in yield and knowledge. Hence a research study was planned and conducted with the aim to analyse and assess the impact of FLD rice on yield, economics conditions and knowledge of rice growers in temperate region of Kashmir.

MATERIALS AND METHODS

Study area

The study was carried out in the temperate region of Indian Kashmir (Figure 1), where agriculture is the mainstay of more than 70% of people. This region is rich in rice culture from centuries. Rice crop plays a significant role in livelihood of people, which is the main staple food crop of the state. Rice covers an area of 2.613 lakh ha in this temperate region with annual production of 5.077 lakh tonnes with an average yield of 19.43 qha^{-1} (Anonymous, 2014). With the aim to increase the productivity of rice in this region, number of high yielding varieties together with site specific technologies has been developed to boost rice production in the region. Due to this intervention, number of landraces and traditional rice varieties grown earlier has been phased out by the cultivation of high yielding varieties (HYV). Due to its high yield potential (8 t/ha), Shalimar Rice-1 (SR-1) is one of the HYV adopted by the farmers especially due to its susceptibility to blast particularly IC-17 and ID-1 races, which are prevalent in rice growing areas of this temperate region. Seed replacement rate in the region is estimated to be 32.54% during 2012-2013 (Anonymous, 2014). The variety has already proved worth through state-wise as well as national FLD programmes conducted since its release. SR-1 has shown 128% increase in the yield in Andhra Pradesh with grain yield of 7.67 q/ha (Anonymous 2012). During 2005, SR-1 has recorded grain yield of 7.5 q/ha against local check of 4.5 q/ha in Kashmir, which reflects potentials well as huge yield gaps. These yield gaps are attributed to lack of awareness among the farming community regarding improved cultivation practice of rice (Singha and Baruah, 2011).

Data collection and sampling techniques used

The study was conducted in four rice productive districts viz., Anantnag, Budgam, Ganderbal and Kulgam of Jammu and Kashmir, in which 240 participants and 240 non-participants of FLD programme, comprising of 12 farmer respondents each from 5 representative villages were selected through stratified random sampling method. The participant farmers constitute those farmers who participated in the FLD rice production technologies by respective Krishi Vigyan Kendra's (Farm Science Centres) during 2007-2012. Yield and economic data of FLDs and farmers practices were collected and analyzed using different parameters as suggested by (Yadav et al., 2004; Sengupta, 1967). Level of knowledge amongst respondent farmers was calculated based on Client Satisfaction Index developed by (Kumaran and Vijayaragavan, 2005) with little modifications to adjust package of practice for rice as recommended by SKUAST (K). Based on thorough discussions with the experts and review of relevant literature, a total of 18 independent variables comprising of socio-personal, socio-economic, psychological, communication and extension system variables, having some bearing on the dependent variables were identified for inclusion in the study. The independent variables represented age (AGE), literacy (LIT), family size (FSZ), occupation (OCP), farm equipments (FIM), operational land holding (OLH), socio economic status (SES), innovative proneness (INP), achievement motivation (AMT), scientific orientation (SOT), social participation (SPT), cosmos-politeness (CPN), extension contact (EXC), exposure to mass media (EDM) and attitude towards farm diversification(AFD) were empirically measured by procedures evolved for the purpose using appropriate scales and scoring procedures developed by earlier researchers. The data was collected through personal interviews and analysed using R Software.

Analytical tools used

Data analysis was carried out by employing appropriate statistical packages. However, below mentioned formulae were used to analyse yield-gaps and economic returns:

$$\text{Extension gap} = \text{yield through demonstration} - \text{Farmers' practice yield}$$

$$\text{Effective gain} = \text{Additional return} - \text{Additional cost}$$

$$\text{Technology gap} = \text{Potential yield of variety} - \text{Demonstration yield}$$

$$\text{Additional return} = \text{Returns through FLDs} - \text{Returns through Famer's practice}$$

$$\text{Incremental B : C ratio} = \text{Additional return} / \text{Additional cost}$$

$$\text{Technology index} = \text{Demonstration yield}) / \text{Potential yield}) \times 100$$

RESULTS AND DISCUSSION

Grain yield

The increase in grain yield under Front Line Demonstrations was 19.25% (Kulgam) to 35.61% (Ganderbal) than farmers practice with mean average of 27.75%. Maximum and minimum yield was recorded in

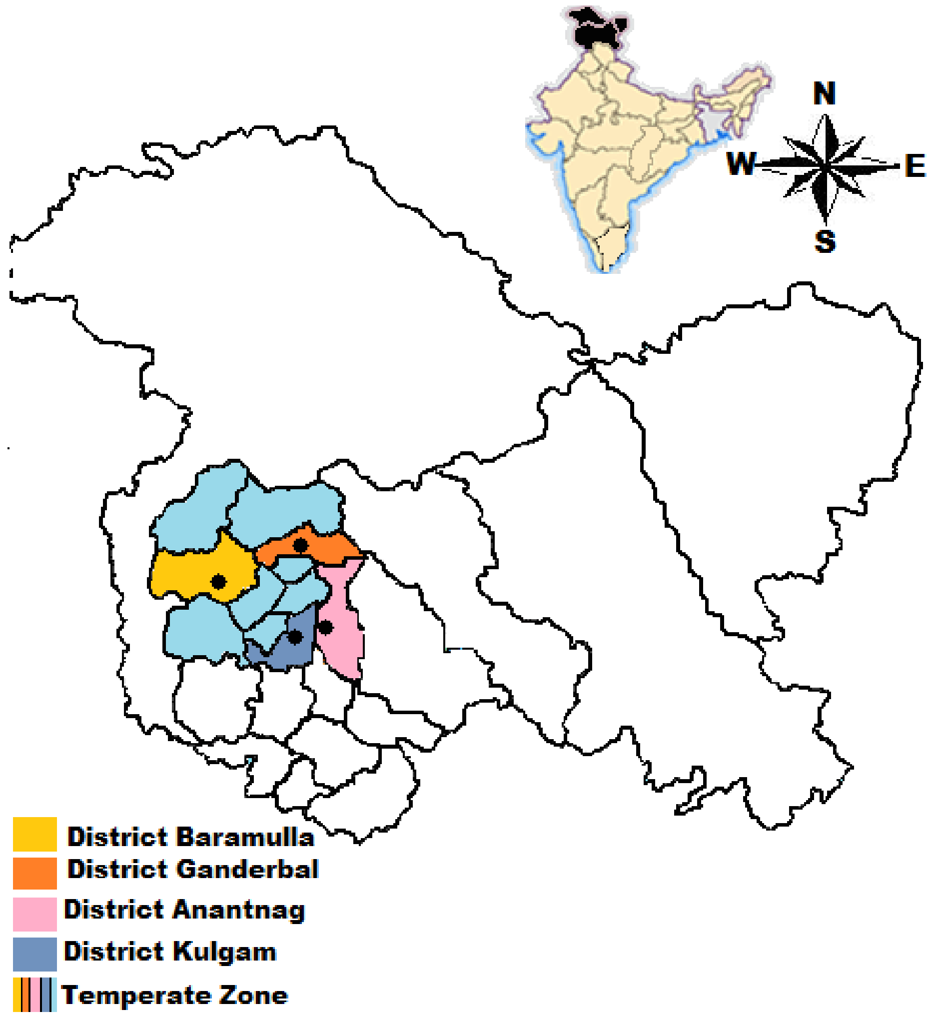

Figure 1. Study area.

Kulgam (56.25 q/ha) and Baramulla (46.92 q/ha) respectively under demonstrations with average demonstration yield of 63.69 q/ha using improved cultivation technology as compared to average yield of 49.99 q/ha using farmers practice (Table 1). Similar yield enhancement in different crops in front line demonstration has amply been documented by (Haque, 2000; Sagar and Chandra, 2003; Singh et al, 2007; Mishra et al., 2009; Kumar *et al.,* 2010; Sheikh et al., 2013; Singh and Sharma, 2004).

Gap analysis

Average extension gap (EG) was 13.70 q/ha which was highest in Ganderbal (16.75%) and lowest in Kulgam 10.83%. Wide technology gaps (TG) were observed in all the districts with average TG of 16.31 q/ha (Table 1). The difference in technology gap during different years could be due to more feasibility of recommended technologies in different districts and variability in climatic conditions. Similarly, the technology index for all the demonstrations were in accordance with technology gap. Higher technology index reflected the insufficient extension services for transfer of technology. The results are in conformance with (Girish et al., 2011).

Economic analysis

Different variables like seed, labour, fertilizers, bio fertilizers and pesticides were considered as cash inputs for the demonstrations as well as farmers practice. An additional average investment of Rs.8046.50/ha resulted effective gain of Rs.29635.75/ha with IBCR of 3.66 (Table 2). Therefore, it can be concluded that FLDs have enhanced the overall grain yield with additional returns as compared to farmers practice. The results confirm the

Table 1. Yield and gap analysis of FLD on Rice at farmers field.

District	No. of demonstrations	Area (ha)	Grain yield (q/ha)		Yield Increase (%)	Extension gap (q/ha)	Technology gap (q/ha)	Technology index
			Participating farmer	Non-participating farmer				
Kulgam	60	24	67.08	56.25	19.25	10.83	12.92	19.26
Anantnag	60	24	65.57	49.75	31.79	15.82	14.43	22.01
Ganderbal	60	24	63.78	47.03	35.61	16.75	16.22	25.43
Baramulla	60	24	58.33	46.92	24.33	11.41	21.67	37.15
Average	60	24	63.69	49.99	27.75	13.70	16.31	25.96

Potential yield of SR-1= 80 q/ha.

Table 2. Economic analysis of front line demonstrations on rice at farmers field.

District	Cost of cash input (Rs./ha)		Additional cost in demonstrations (Rs./ha)	Sale price of grain (MSP) (Rs./qt)	Total returns (ha)		Extra returns	Effective gain	IBCR
	FP	Demo			FP	Demo			
Kulgam	45700	52660	6960	2750	184470	154688	29783	22823	3.28
Anantnag	42241	50602	8361	2750	180318	136813	43505	35144	4.20
Ganderbal	47392	55948	8556	2750	175395	129333	46063	37507	4.38
Baramulla	46563	54872	8309	2750	160408	129030	31378	23069	2.78
Average	45474	53520.5	8046.5	2750	175147.8	137466	37682.25	29635.75	3.66

IBCR = Incremental Benefit: Cost ratio; FP = Farmers practice. Demo, demonstrations.

findings of FLDs on Rice by Lathwal (2010) and Dayanand et al. (2011).

Knowledge about improved rice production practices

Knowledge level of respondent farmers on different parameters of improved rice production technologies were measured and compared by applying dependent't' test. It could be seen from the Table 3 that participant farmer's knowledge was found in the range of medium to high as compared to low to high in non-participating farmers. Among participating farmers maximum farmers (55%) in district Kulgam had highest level of knowledge followed by 46% in Ganderbal. Minimum knowledge level (28.3%) was recorded in district Anantnag amongst participating farmers. In respect of non-participating farmers, maximum (41.8%) farmers from district Anantnag had low level of knowledge. The results are at par with (Singh and Sharma, 2004) and (Singh et al., 2007). It means there was significant increase in knowledge level of the farmers due to frontline demonstration. This shows positive impact of frontline demonstration on knowledge of the farmers. The results so arrived might be due to the concentrated efforts made by the field functionaries.

Correlates of knowledge levels of improved practices of rice

In order to highlight the factors which are related to knowledge levels of improved practices of rice, co-relation analysis was carried out between selected variables of farmers and their knowledge behaviour using statistical package 'R Software' and the correlation coefficients are given in Table 4.

From correlation coefficients, it is clear that the age, literacy, operational land holding, extension contact, social participation, achievement motivation and attitude towards land diversification were significant and positive bearing on the knowledge levels of participating farmers towards improved practices. However, negative influence was showed by 'exposure to different media' variable. Negative influence of participating farmers about knowledge may be due to media usage for entertainment and personal ambitions. These results implied that high levels of knowledge holders of improved practice of rice would be educated farmers having good extension contact, ability to diversify farms and frequent social participation. Hence educated participant farmers had higher knowledge about improved practices. The results are similar to those of Amol (2006), (Ankulwar et al. (2001) and Singh et al. (2010).

Table 3. Knowledge level comparison between farmers about improved practices.

	Anantnag				Kulgam				Ganderbal				Baramulla			
	PF (N=60)		NPF (N=60)		PF (N=60)		NPF (N=60)		PF (N=60)		NPF (N=60)		PF (N=60)		NPF (N=60)	
Mean (%)	69.26		24.07		77.22		24.81		65.56		24.26		59.44		23.52	
Standard deviation	13.78		16.13		13.94		14.23		18.94		13.49		16.34		13.43	
Range	55.56-88.89		11.11-66.67		55.56-88.89		11.11-66.67		44.44-88.89		11.11-55.56		44.44-88.89		11.11-55.56	
Category	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No**	**%**	**No.**	**%**	**No**	**%**
Low (<Mean - SD)	-	-	25	41.8	-	-	19	31.7	-	-	19	31.7	-	-	22	36.7
Medium (between mean ± SD)	43	71.7	19	31.7	27	45.0	24	40.0	32	53.3	25	41.7	39	65.0	22	36.7
High (> mean + SD)	17	28.3	16	26.7	33	55.0	17	28.3	28	46.7	16	26.7	21	35.0	16	26.7

Table 4. Correlation coefficients of knowledge levels of improved rice production amongst farmers.

Variables	Correlation coefficient							
	Participating farmers (N=240)				Non-participating farmers(N=240)			
	Anantnag	Baramulla	Ganderbal	Kulgam	Anantnag	Baramulla	Ganderbal	Kulgam
AGE	0.622*	0.499*	0.379*	0.513*	0.346*	0.425*	0.334*	0.428*
LIT	0.313*	0.423*	0.351*	0.058	0.372*	0.372*	0.374*	0.436*
FSZ	-0.201*	-0.124	-0.086	0.106	0.053	-0.098	0.202	0.041
OLH	*0.190	0.339*	0.308*	0.430*	0.099	0.259*	0.289*	0.053
FIM	-0.112	0.025	0.175	-0.253*	0.033	0.078	0.066	0.179*
SES	-0.056	0.043	0.257*	-0.052	0.221*	-0.250*	0.006	-0.140
OCP	0.030	-0.075	0.108	0.016	0.093	-0.148	0.002	-0.040
AMT	-0.002	0.251*	0.251*	-0.228*	0.150	0.104	0.179	-0.067
IPN	-0.126	0.074	0.111	0.064	-0.213*	-0.019	-0.063	-0.049
CPN	0.009	0.022	0.023	-0.011	-0.015	-0.021	0.067	0.133
SOT	0.126	-0.470*	-0.303*	0.071	-0.231*	-0.431*	0.040	-0.011
SPT	0.130*	0.299*	0.310*	-0.019	-0.151	-0.122	0.198	-0.327*
EXC	0.394*	0.506*	0.396*	0.439*	0.218*	0.623*	0.308*	0.361*
EDM	-0.160*	-0.507*	-0.269*	-0.534*	0.032	0.139	-0.075	0.131
AFD	0.397*	0.499*	0.470*	0.479*	0.127	0.148	0.054	-0.028

*Significant at 0.05 level of probability.

Regression analysis

In order to assess the contribution of various independent variables to the variation in the knowledge level amongst respondent farmers, regression analysis of the dependent variable was done. A regression equation was fitted with the dependent variable of knowledge level scores of improved practices of rice and fifteen independent

Table 5. Regression coefficients of knowledge levels of farmers.

Variables	Regression coefficient							
	Participating farmers (N=240)				Non-participating farmers(N=240)			
	Anantnag	Baramulla	Ganderbal	Kulgam	Anantnag	Baramulla	Ganderbal	Kulgam
Constant	7.066	4.660	2.85*3	4.639	0.257	0.305	-0.498	1.073
AGE	3.996*	0.436	0.872	2.183*	2.726*	0.794*	1.923*	1.054*
LIT	2.776*	2.319*	1.069	0.595	2.970*	1.900*	1.996*	4.096*
FSZ	-1.099	-0.862	0.113	0.528	1.190	-0.211	1.767*	-0.343
OLH	1.469	2.107*	0.843	1.929*	1.321	1.447	0.795	-0.386
FIM	-1.793	-0.755	2.069*	-0.516	0.097	0.176	0.062	-0.409
SES	-1.280	-0.807	2.115*	1.279	0.339	-1.521	0.395	0.137
OCP	1.479	0.524	0.342	1.192	0.445	-0.431	0.642	-1.549
AMT	-1.193	-0.048	0.319	-1.312	-0.504	0.429	0.672	-0.074
IPN	-0.488	-0.910	-0.012	0.764	-1.801	1.198	-0.492	-0.742
CPN	-0.679	1.209	-1.216	0.737	0.237	-0.011	0.126	1.266
SOT	0.835	-1.152	-1.244	-0.217	-2.288*	-0.879	0.215	-0.741
SPT	-0.755	1.275	-0.846	-0.469	-0.179	-0.782	1.342	-2.708*
EXC	2.267*	2.040*	2.410*	2.534*	0.563	4.402	1.77*9	3.607*
EDM	-2.176*	-2.174*	-2.333*	-1.934*	1.242	0.290	-0.802	1.454
AFD	1.952*	3.392*	*2.415	2.070*	-0.557	0.902	1.048	-0.594
R	0.815	0.832	0.764	0.794	0.664	0.776	0.638	0.758
R^2	0.664	0.692	0.584	0.630	0.441	0.603	0.407	0.575
F	5.80**	6.61**	6.68**	5.00**	2.32	4.46	2.02	3.98

*Significant at 0.05 level of probability; ** significant at 0.01 level of probability.

variables. The results of the analysis are presented in Table 5.

A perusal of the results presented in Table 5 indicates that about 22, 9, 18 and 6% variation between participant farmers and non-participant farmers in Anantnag, Baramulla, Ganderbal and Kulgam respectively exists with respect to knowledge of improved rice production practices, which were explained by the independent variables included in the regression equation. Among participating farmers F value had significant 0.01 level of probability throughout the study area. This indicates that the independent variables included in the study were appropriate as they could explain large variance in the dependent variable. Also a cursory look at the table reveals that only five variables viz., age, literacy, extension contact and farm diversification could contribute significantly to the variance and predicting different knowledge levels of improved Rice production practices amongst participant farmers. Moreover, among non-participating farmers only age and literacy was found to contribute significantly. Thus it can be concluded that FLD on Rice has greatly influenced on the knowledge of the participant farmers than non-participant farmers.

Conclusion

Concept of FLD has been instrumental in enhancing yield to the extent of 27.75% with IBCR of 3.66, which is very encouraging. However extension and technology gap of 13.70 and 16.31 q/ha respectively exists still, due to low to medium knowledge levels of improved production practices among majority farmers. Indeed knowledge dissemination through FLD programme has increased level of knowledge among participant farmers as compared to non-participant farmers to a significant level. This shows positive impact of frontline demonstration on yield and knowledge of the farmers. The results so arrived might be due to the concentrated efforts made by the field functionaries. The results of regression analysis revealed that FLD programme has helped in contributing to enhancement of improved rice production technology. This can be seen as a positive indicator for formulating an objective specific and extensive FLD programme to train and educate farmers about improved rice production practices through 'working by doing' and 'doing by learning' for ensured higher Rice production in the region.

Conflict of Interest

The authors have not declared any conflict of interest.

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Scientific Research and Essays

Full Length Research Paper

Purification and biochemical characterization of extracellular manganese peroxidase from *Ganoderma lucidum* IBL-05 and its application

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In this study an extracellular manganese peroxidase (MnP) was isolated from culture filtrate of an indigenous fungal strain *Ganoderma lucidum* IBL-05 under static conditions using wheat bran as substrate. The enzyme was purified by applying successively ammonium sulphate precipitation, dialysis, ion exchange and gel filtration chromatographic techniques. Purification procedure resulted in 3.43-fold purification with corresponding specific activity of 539.59 Umg-^{1}. The purified MnP elucidated single band in 43 kDa region on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The purified MnP showed optimum activity at pH 5 and 40°C temperature. The K_m and V_{max} for MnP toward $MnSO_4$ as a substrate were found to be 65.5 mM and 640 UmL^{-1}, respectively. It was observed that MnP activity enhanced by Mn^{2+} and Cu^{2+} and inhibited in the presence of Zn^{2+}, Fe^{2+}, EDTA and Cysteine to various extents with Hg^{2+} (most inhibitory). The purified MnP efficiently catalyzed the transformation of different synthetic textile dyes (Sandal-reactive dyes). Characterization revealed that MnP isolated from *G. lucidum* have potential to be used for myriad industrial and biotechnological applications.

Key words: Manganese peroxidase, *Ganoderma lucidum* IBL-05, purification, characterization, kinetics, dye decolorization.

INTRODUCTION

Significant efforts have been made on filamentous fungal biotechnology in recent years in order to obtain value added products such as enzymes, chemicals, liquid biofuel, secondary metabolites and spores (Reina et al., 2013). White-rot basidiomycetes are the principal organisms that secrete a unique set of extracellular oxidoreductases comprising lignin peroxidase (LiP; Ec1.11.1.14), manganese peroxidase (MnP; EC 1.11.1.13) and laccase (Lac; EC 1.10.3.2). These catalysts are known with remarkable potential to depolymerize lignin and various environmental pollutants such as polycyclic aromatic hydrocarbons, synthetic dyes and cholorophenols (Du et al., 2015). Different white rot fungi (WRF) secrete different set of lignin mineralizing

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enzymes (LMEs) and each fungus secretes one or more of the three enzymes essential for lignin degradation (Levin et al., 2008). Besides, some versatile peroxidases (VP; EC.1.11.1.16) with combined LiP and MnP catalytic properties have also been reported (Morgenstern et al., 2008).

Due to their interesting catalytic properties, MnPs have gained considerable interest in various industrial areas. The most intensively studied applications have included bioremediation, biomass delignification, oxidation of organic pollutants, bio sensing, textile, animal feed, cosmetics, detergent manufacturing, paper and pulp, transformation of antibiotics and steroids etc (Sylvia et al., 2015). MnP (EC 1.11.1.7) is a lignin-modifying glycoprotein synthesized by wood-colonizing basidiomycetes during secondary metabolism. It catalyzes phenolic compounds to phenoxy radicals by oxidation of Mn^{2+} to reactive Mn^{3+} in H_2O_2 dependent enzymatic reaction (Ferhan et al., 2012). However, some low molecular weight mediators can increase the substrate range of MnPs to non-phenolic structures (Giardina et al., 2000). Further, many proteins acting synergistically with MnPs, has expanded the role of these enzymes in fungal lignolysis (Hilden et al., 2000).

Among WRF, *Ganoderma lucidum* is considered as the most commonly used organism in biodegradation studies due to its good ligninolytic properties, fast growth potentials, and environmental-friendly nature (Batool et al., 2013). Keeping in view the extensive industrial applications of MnP, the present study was accomplished with an objective to purify and characterize the extracellular MnP from an indigenous WRF strain *G. lucidum* IBL-05 and then tested it for its ability to decolorize different textile dyes.

MATERIALS AND METHODS

Fungal culture and inoculum development

For inoculum development, the indigenously isolated *G. lucidum* IBL-05 strain was grown in Kirk's basal nutrient medium (Tien and Kirk, 1988) in Erlenmeyer flask (250 ml) that was supplemented with Millipore filtered 1% glucose. Prior to sterilization, the medium was adjusted at pH 4.5 with 1 M NaOH/1 M HCl and inoculated with spores of *G. lucidum* IBL-05 from slant culture. The inoculated flask was incubated (120 rpm) at 30°C for 5 to 7 days to get homogenous spore suspension of the fungus (1×10^6-10^8 spores/ml) and used as inoculum.

Production and extraction of MnP

Triplicate conical flasks containing 5 g semi-solid wheat bran were autoclaved and inoculated with 5 ml homogenous inoculum (Ramzan et al., 2013). The inoculated flasks were allowed to ferment at 30°C in a temperature controlled incubator for 5 to 7 days at pH 4.5. After growth, sterile samples were taken after every 24 h and MnP activity was monitored. When MnP activity was peaked to a maximum level, the fermented biomass was harvested by adding 100 ml distilled water, filtered, centrifuged (Ependorf 5415C, Germany) and clear supernatant was assayed for MnP enzyme and stored at 4°C in refrigerator for further characterization. All experiments were carried in triplicate to avoid the discrepancy in results.

Determination of MnP activity and protein contents

The MnP activity was determined by monitoring the formation of Mn^{3+}-malonate complexes at 270 nm (ε_{270} = 11570 M cm^{-1}, Wariishi et al., 1992). Assay mixture contained 1 ml of $1x10^{-3}$M $MnSO_4$, 1 ml of $50x10^{-3}$M sodium malonate buffer (pH 4.5) and 0.5 ml of H_2O_2 in combination with 0.1 ml enzyme solution. Absorbance of each sample was measured Spectrophotometrically (HALO DB-20). The protein concentration was estimated according to Bradford method (1976) using bovine serum albumin (BSA) as a standard.

Purification of MnP

All the purification steps were conducted below 4°C. Briefly, crude MnP extract obtained from 5 days old culture of *G. lucidum* IBL-05 was centrifuged at 3,000 × g for 15 min. The cell-free supernatant was saturated (up to 35%) by gradual addition of ammonium sulphate and kept for overnight at 4°C. The resulting precipitate, thus obtained was recovered (3,000 × g for 20 min at 4°C) and supernatant was again saturated by adding ammonium sulfate (up to 65%), allowed to stand overnight at 4°C, centrifuged and pellets were dissolved in 50 mM Sodium Malonate buffer (pH 4.5). The solution was kept in dialysis bag and dialyzed against the same buffer, after sealing it securely and finally, dialyzate was freeze dried. The dialyzate obtained was submitted to ion-exchange chromatography using diethyl amino ethyl (DEAE) cellulose column, equilibrated with phosphate buffer (100 mM; pH 6.5) for 24 h and eluted with 0 to 1.0 M linear gradient of NaCl at a flow rate of 0.5 ml/min. The MnP active fractions were pooled and loaded onto Sephadex-G-100 column (10×300 mm). Up to 30 positive fractions (1 ml) with flow rate of 0.3 ml/min were collected and absorbance was measured at 280 nm (Zeng et al., 2013).

Gel electrophoresis and staining

The MnP purification was confirmed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) following the method of Laemmli (1970). The molecular mass of MnP was approximated after gel staining with Coomassie Brilliant Blue G-250 followed by calibration against standard protein markers (Sigma, USA), ranging from 17-170 kDa.

Characterization of purified MnP

Effect of pH on MnP activity was investigated by incubating enzyme for 15 min at varying pH values (3 to 10). For stability studies the enzyme was pre-incubated at varying pH for 1 h. MnP activities were also determined at various temperatures between 30 and 70°C under optimal pH values. The enzyme was incubated for 15 min at varying temperatures before running the enzyme assay. For thermal-stability the enzyme was incubated at different temperatures for 1 h without substrate before carrying out MnP assay. K_m and V_{max} for the purified enzyme were calculated using the Lineweaver Burk transformation of Michaelis-Menten equation. The standard quartz cuvettes of 1 mm path length were used to calculate the values of kinetic parameters. The enzyme was incubated for 15 min at 30°C in sodium malonate buffer of pH 4.5 before carrying out standard enzyme assay protocol. Lineweaver-Burk (Double reciprocal) plot was generated with

Microsoft Excel Windows updated version 7 via nonlinear regression analysis using different concentrations (0.1 to 1.0 mM) of manganese sulphate as substrate at optimum pH 5 and 40°C temperature. The effects of various modulators (Mn^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Hg^{2+}, K^{+}, Ethylene diamine tetra acetic acid (EDTA) and cysteine) on enzyme activities were tested in the concentration range of 5 to 20 mM. Enzyme activities measured without any modulator was considered as 100% (Zeng et al., 2013).

Dyes and decolorization studies

Three dyes namely Sandal-fix Red C_4BLN (λmax: 540 nm), Sandal-fix Turq Blue GWF (λmax: 664 nm) and Sandal-fix Black CKF (λmax: 598 nm) were used to investigate the decolorization potential of purified MnP. For this, MnP solution was transferred to triplicate Erlenmeyer flasks (500 ml) containing 100 ml of individual dye solution (0.1 mg/ml) in combination with Na-malonate buffer (50 mM; pH 4.5). Flasks were incubated in rotary shaker (Sanyo-Gallenkamp, UK) at 40°C for 12 h. The flasks content were filtered, centrifuged (8,000 × g, 10 min) and dye removal was monitored spectrophotometrically (HALO DB-20) at respective wavelengths. The decolorization efficiency was calculated using relation (Equation 1).

$$\text{Decolorization } (\%) = \frac{Ai - At}{Ai} \times 100 \qquad (1)$$

Where, A*i* and A*t* are representing absorbance at zero and time *t*.

Statistical analysis

Mean and standard deviation (SD) of the results based on three independent experiments were calculated using Microsoft Excel-software (Microsoft) and the standard error (SE) values were displayed as Y-error bars in figures.

RESULTS AND DISCUSSION

Production of MnP

In this study, a locally isolated fungal strain, *G. lucidum* IBL -5was exploited for ligninolytic enzyme (Lip, MnP and laccase) production potential in solid state medium of wheat bran under pre-optimized growth conditions such as moisture, 50%; substrate, 5 g; pH, 5.5; temperature, 30°C; carbon source, 2% glucose; nitrogen source, 0.02% yeast extract; C:N ratio, 25:1; fungal spore suspension, 5 ml and fermentation time period, 5 days (Ramzan et al., 2013). The enzyme extract contained 576.3, 717.7 and 323.2 UmL^{-1} of LiP, MnP and laccase, respectively.

Purification of MnP

A sequential four-step purification procedure involving ammonium sulphate fractionation, dialysis, DEAE-cellulose ion exchange and G-100 sephadex gel permeation chromatography was employed for the purification of MnP. A mixture of crude ligninolytic extract obtained from five days incubated culture (300 ml) of *G. lucidum* on wheat bran was purified to homogeneity as summarized in Table 1. The MnP was completely salted out at 65% saturation with $(NH_4)_2SO_4$ to 1.73 fold purification with specific activity of 273.01 U/mg. After ammonium sulphate precipitation, the crude extract was applied to ion exchange chromatography. The elution pattern on the DEAE-cellulose column showed two protein peaks at 280 nm (Figure 1). The fractions with high MnP activities were pooled, concentrated and loaded onto a Sephadex G-100 column. In Figure 2 it was clearly indicated that the MnP eluted in a single prominent peak on gel filtration chromatography. At the end of fourth purification step, the enzyme had been 3.43-fold purified with corresponding specific activity of 539.59 U/mg. The purified MnP appeared as a single band in 43 KDa region on SDS-PAGE analysis (Figure 3), suggesting that the enzyme was a monomeric protein. The MnPs also secreted in a multiple isoform with distinct structural configuration and molecular mass. The molecular masses of MnP vary from 32 to75kDa (Asgher et al., 2013).

Characterization of purified MnP

Effect of pH on MnP activity and stability

The pH profile for purified MnP has been illustrated in Figure 4. The MnP displayed maximum activity at optimum pH 5; beyond this pH value a marked decreasing trend in the activity was observed. Moreover, the purified MnP was fairly stable over a wide range of pH (4-6) at incubated time of 1 h, pH above 6 caused inactivation of the enzyme irrespective of incubation time. In previous studies, maximum activity of MnP from different WRF has been reported in the pH range of 4.5 to 6.5 (de Oliveira et al., 2009). MnP isolated from solid-state culture of corncobs by *Lentinula edodes* exhibited optimum activity at pH 4.5 (Boer et al., 2006); whereas, MnP from *S. commune* IBL-06 was optimally active at pH 5 (Asgher et al., 2013).

Effect of temperature on MnP activity and stability

The MnP activity against temperature curve shown in Figure 5, the plot indicated that initial rise of temperature up to 40°C increased the activity as well as stability of the MnP. Further rise of temperature caused decrease of activity and stability as well due to denaturation of enzyme at elevated heat. The optimum temperature for purified MnP was found to be 40°C. The purified MnP showed fascinating thermal-stability up to 50°C without dropping much of its activity, which would be an attractive and desirable feature for a variety of industrial processes. However, MnP lost almost 50% of its activity when

Table 1. Purification summary of MnP produced by *G. lucidum* IBL-05 in solid state fermentation.

Step	Purification steps	Total volume (ml)	Enzyme activity (U ml^{-1})	Protein contents (mg ml^{-1})	Specific activity (U mg^{-1})	Purification fold
1	Crude enzyme	300	717.4	4.34	132.32	1
2	Ammonium Sulphate ppt.	22	613.7	2.45	273.01	1.73
3	Dialysis	21	598.09	2.13	390.91	1.95
4	DEAE-cellulose	12	584.3	1.68	420.34	2.78
5	Sephadex G-100	9	569.6	0.9	539.59	3.43

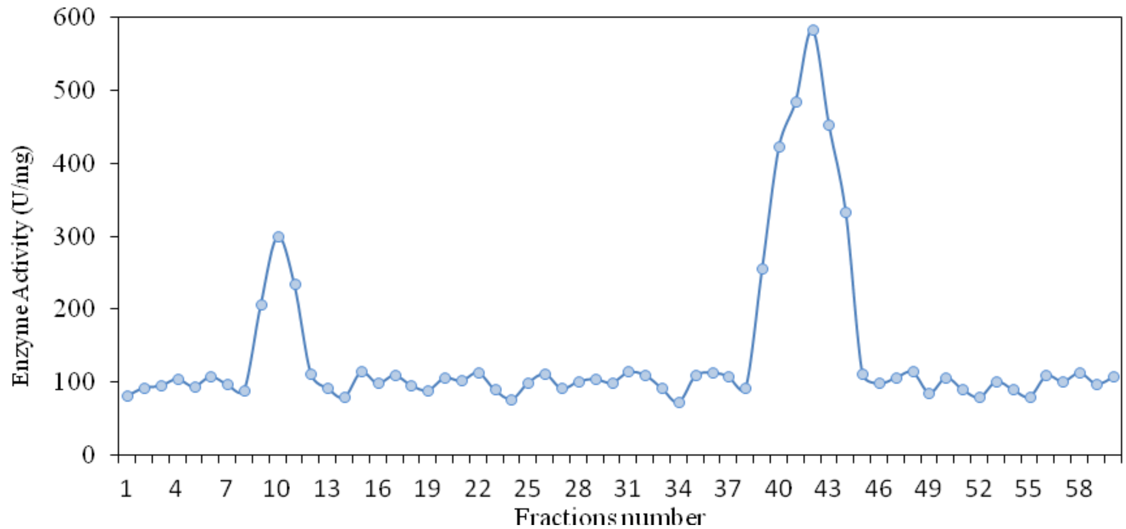

Figure 1. Ion exchange chromatographic purification profile for MnP from *G. lucidum*.

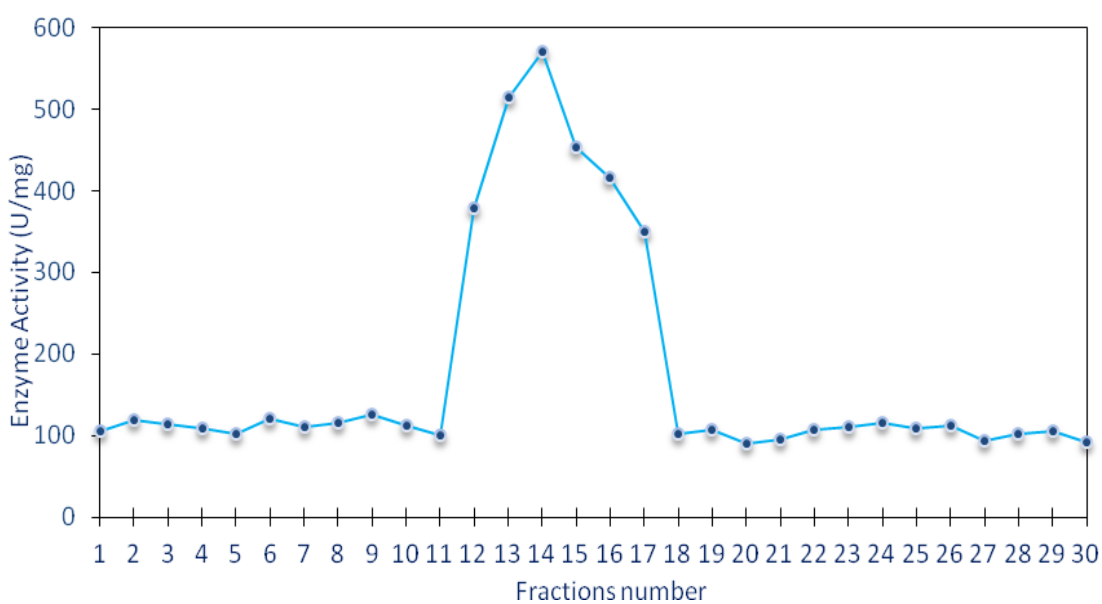

Figure 2. Gel filtration chromatographic purification profile for MnP from *G. lucidum*.

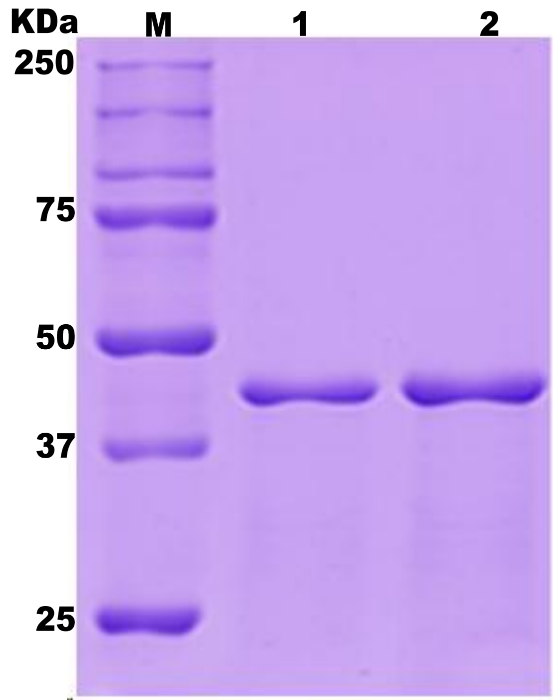

Figure 3. SDS-PAGE of MnP produced from *G. lucidum* IBL-05. The molecular mass of the purified MnP was estimated in comparison to standard protein marker, 25-250 kDa; (Sigma, USA). The protein bands were visualized by staining with Coomassie Brilliant Blue G-250.

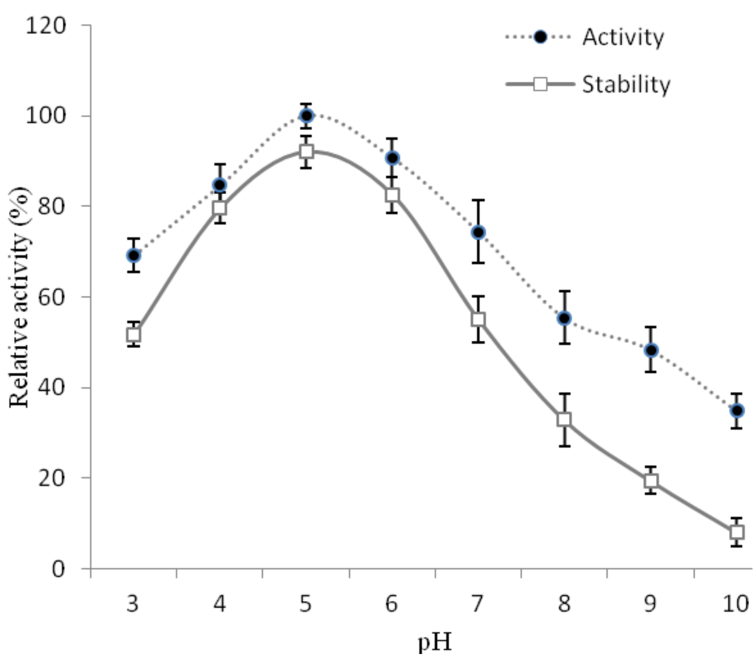

Figure 4. Effect of pH on the activity and stability of MnP. The results presented arethe means of three independent experiments and the bars represent the standard deviation of the means.

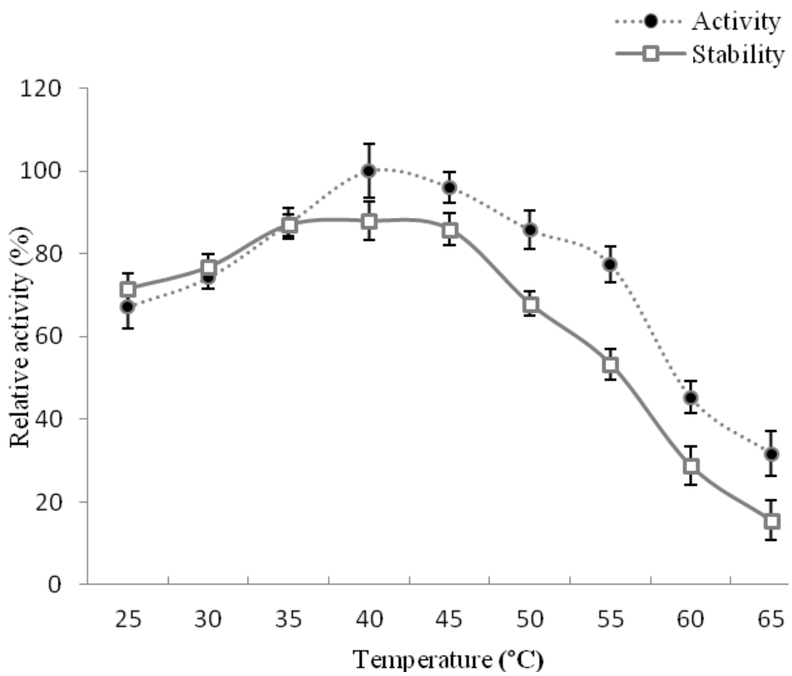

Figure 5. Effect of temperature on the activity and stability of MnP. The results are the means of three replicates and the bars represent the standard deviation of the means.

incubated at 55°C for 1 h. The MnP isolated from different WRF demonstrated optimum temperature around 40 to 60°C (Hakala et al., 2005). The MnP from WRF strain, *Irpex lacteus* was stable in the temperature range of 30 to 40°C (Shin et al., 2005), while *Rhizoctonia* sp. isolated MnP was deactivated over 55°C (Cai et al., 2010). The MnP2 isozyme from *Lentinula edodes* showed thermal-stability up to 40°C (Boer et al., 2006).

Determination of kinetic constants *Km* and *Vmax*

The K_m and V_{max} values were calculated by intercepting line on X-axis and Y-axis of the reciprocal plot, respectively, using different concentration (0.1 to 1.0 mM) of $MnSO_4$ as assay substrate (Figure 6). At 0.5 mM $MnSO_4$ concentration, the maximum MnP activity (859 UmL^{-1}) was furnished with K_m 65.64 µM and V_{max} 640 UmL^{-1} using non-linear regression analysis at optimum pH and temperature. The difference in K_m values of MnP from different reported fungal species might be due to the genetic variations and substrate specificities among various species. The interaction of enzyme with its substrate was indicated through K_m values and a lower K_m value reflect high affinity of enzyme for its substrate and higher V_{max} indicated that small amount of enzyme can convert substrate into the product (Asgher et al., 2014).

Effect of various modulators on activity of MnP

In order to identify the nature of enzyme, the effects of various organic compounds and metal ions as possible inhibitors and activators on MnP activities were studied (Table 2). The results revealed that Mn^{2+} and Cu^{2+} enhanced the activity of MnP at all tested concentrations. The metal ion Mn^{2+} showed the most significant role to activate *G. lucidum* MnP, which was consistent with the findings of Boer et al. (2006). In addition, low concentrations of Co^{2+} (5 mM) drive up MnP activity but at higher concentration of Co^{2+} (20 mM) a slight inhibition was observed (91%). Elevated concentrations of K^+ enhanced the MnP activity (117%) but lower K^+ concentrations (5 mM) did not exert any effect on MnP activity. On the other hand, $Zn^{2+}$and Fe^{2+} partially inhibited the MnP, whereas MnP activity was strongly inhibited by Ethylene diamine tetra acetic acid (EDTA) and cysteine and even Hg^{2+} fully inactivated MnP enzyme. The MnP from *P. chrysosporium* was inhibited by NaN_3, *β*-mercaptoethanol and dithreitol, whereas co-oxidants such as glutathione, un-saturated fatty acids and Tween 80, significantly enhanced the MnP activity (Urek and Pazarlioglu, 2004). *Trichophyton rubrum* LSK-27 MnP was entirely inhibited by Hg^{2+}, while Fe^{3+}, Ca^{2+} and Ni^{2+} did not show any inhibitory effect on enzyme activity (Boer et al., 2006).

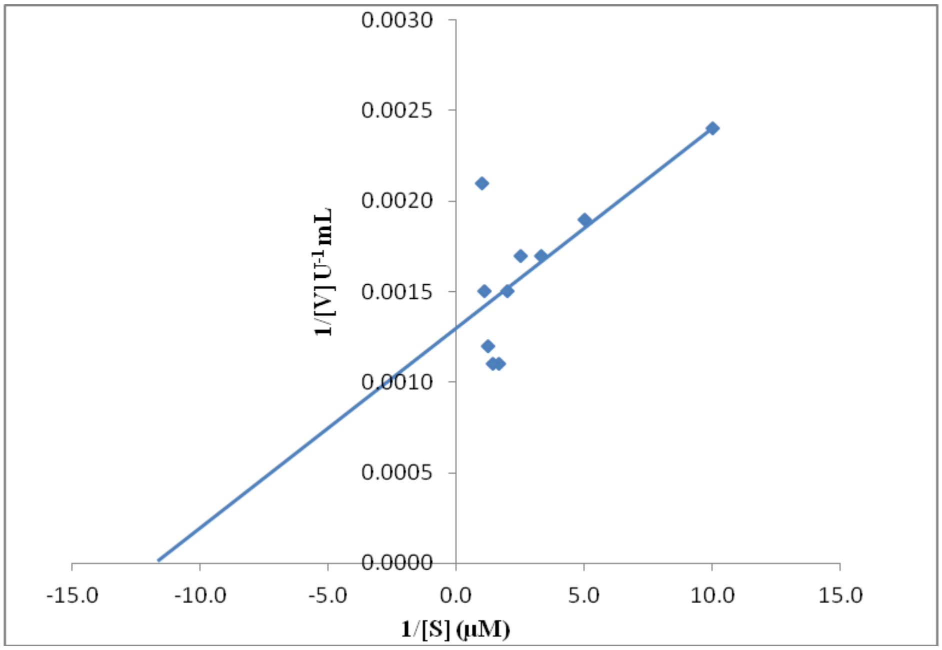

Figure 6. Lineweaver Burk Plot for determination of K_m and V_{max} for purified MnP.

Table 2. Effects of various modulators on the activity of purified MnP from *G. lucidum*.

Modulators	Concentration (mM)	Relative activity of MnP (%)
Mn^{2+}	5	119±6.4
	10	139±5.3
	20	147±3.7
Zn^{2+}	5	89±2.8
	10	71±2.5
	20	58±4.2
Co^{2+}	5	109±5.1
	10	103±4.6
	20	91±4.7
Cu^{2+}	5	114±4.5
	10	117±3.9
	20	128±4.9
Fe^{2+}	5	82±1.4
	10	57±2.4
	20	42±4.2
Hg^{2+}	5	14±0.9
	10	7.6±0.13
	20	3.3±0.29
K^{2+}	5	101±4.0
	10	109±3.9
	20	117±5.3

Table 2. Contd.

EDTA	5	45±1.9
	10	32±2.3
	20	27±2.4
Cysteine	5	38±1.7
	10	21±2.1
	20	9±0.73

All data are expressed in mean value ±standard deviation.

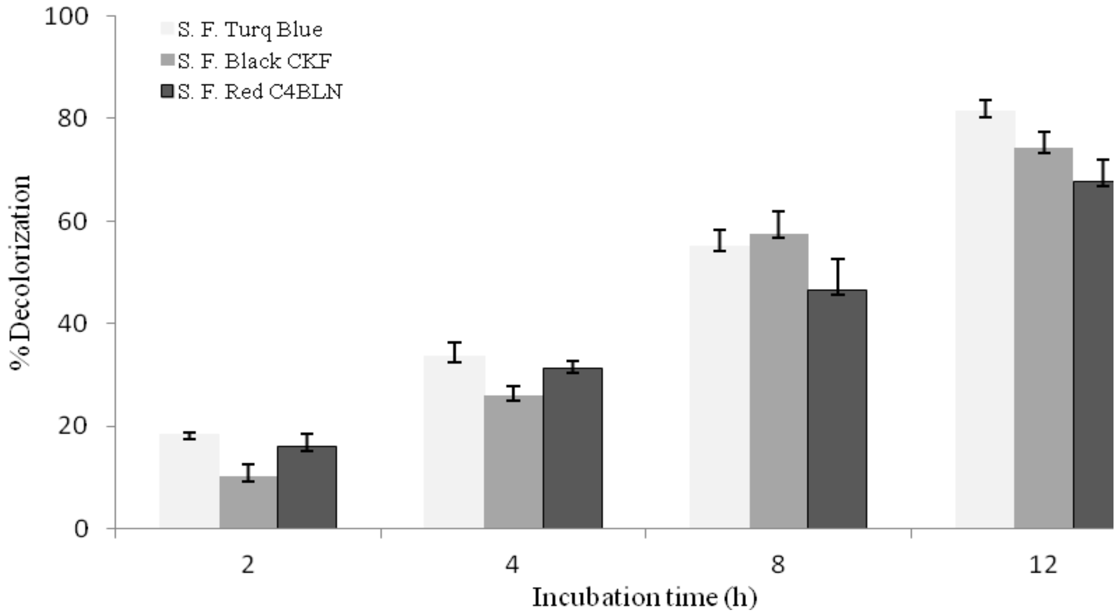

Figure 7. Percentage decolorization of textile dyes by purified MnP from *G. lucidum* IBL-05. The results are the means of three replicates and the bars represent the standard deviation of the means.

Decolorization of different textile (synthetic) dyes by purified MnP

The dye-decolorizing potential of purified MnP from *G. lucidum* was demonstrated for different synthetic dyes (Sandal-reactive dyes) at different time periods. From data in Figure 7 it can be seen that MnP caused maximum decolorization of S.F. Turq Blue dye to 81.3%, followed by S.F. Black CKF to 74.2% and S. F. Red C_4BLN dye to 67.8% within 12 h of incubation period. The MnP was more effective for decolorization of different textile dyes including Remazol brilliant blue R (RBBR), Congo red, methylene blue and ethyl violet (Bazanella et al., 2013). The findings correlated with previous investigations (Cheng et al., 2007), which confirmed that MnP has remarkable catalytic potential to degrade and mineralize dyes and colored effluents. The peroxidases caused dye degradation by generating highly active free radicals such as Mn^{3+}, lipid, hydroxyl, and peroxy-radicals (Hofrichter, 2002). However, the dyes are not uniformly susceptible to biodegradation because of the structural diversity (Murugesan et al., 2006).

Conclusion

The ion exchange and gel filtration column chromatography techniques were used to purify MnP enzyme from *G. lucidum* up to 3.43-fold. The molecular weight of purified MnP was determined to be 43 kDa from SDS-PAGE analysis. Purified MnP showed encouraging activity and stability at their optimal pH and temperature. Further, the purified MnP possesses effective dye decolorization capability, indicating a useful tool for bioremediation purposes. The high level MnP production and its novel catalytic features suggest its suitability for industrial and biotechnological applications. Nevertheless, further molecular approaches are needed for improving its catalytic and thermal-stability characteristics that will be the focus of future research.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENT

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Scientific Research and Essays

Full Length Research Paper

Effect of magnesium fertilization on some plant nutrient interactions and nut quality properties in Turkish hazelnut (*Corylus avellana* L.)

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Plant nutrient interactions and fertilization is one of the most important factors affecting the quality of hazelnuts. This study aimed to determine the relationships between some plant nutrients and some hazelnut quality criteria and yields. The trial was carried out at Tombul hazelnut orchards in Hazelnut Research Station between 2009 and 2011. The trial was designed as randomized complete blocks of magnesium nutrient at five different doses and three replications per treatment. Hazelnut orchards were fertilized with 0, 100, 150, 200 and 250 kg ha^{-1} magnesium in each year. Magnesium fertilization significantly affected hazelnut yield and quality. However, increasing amounts of magnesium fertilizer in the soil, where they cause significant nutrient interactions were identified. The magnesium content of the soils significantly increased to 260.30 mg kg^{-1}; however, because of the recent interactions, the available phosphorus and potassium of the soil decreased to 84.28 and 298.24 mg kg^{-1} significantly. The total yield was increased to 1747.05 kg ha^{-1} with 150 kg ha^{-1} magnesium fertilization. The protein quantity in the kernel was not changed but there was important increased in oil content, kernel ratio, shelled and kernel nut weight and healthy nut amounts, and also was decreased in the amount of empty and wrinkle nuts were identified. Although the total yield increased with increasing amount of magnesium fertilizer (200 and 250 kg ha^{-1}), especially the phosphorus and potassium interactions were increased in the soil, and also some nut quality properties such as kernel ratio, shelled and kernel weights, total oil, healthy nuts, empty and wrinkle nuts were be getting worse.

Key words: *Corylus avellana* L., fertilization, interactions, nutrients, quality.

INTRODUCTION

It is known that the 1.93% of the earth's crust is composed of magnesium. The magnesium appears in the earth's crust in the form of carbonate, silicate, sulfate, chloride and it is commonly found in the important

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minerals like biotite, dolomite, magnesite etc. (Kacar, 1984). In spite of the high amount of magnesium in the earth's crust, today there is often a lack of magnesium in several agriculture fields. Aktaş (1994) stated that the probability of magnesium deficiency is high in the especially the low percent of the base saturation and cation exchange capacity of the sandy and acidic soil. Kacar (1984) indicated that lack of magnesium is likely to be seen in the coarse-textured soil which is found in the areas that get heavy rain and the constant calcification of this kind of soil causes the lack of magnesium. Kacar and Katkat (2007) reported that the fact that chemical fertilizers which are produced with developing technology do not include magnesium, more products are harvested with recently developed varieties of plants and more nutritional elements are extracted from the soil increase the magnesium requirements. Excess nitrogen and potassium fertilizer is applied in soil solution comprising a high concentration with K^+ and NH_4^+ ions with Mg^{2+} ions by competing plants that inhibit the uptake of Mg^{2+}.

Hazelnut agriculture in Turkey, with production areas about 690.000 ha, is located in the West, Middle and East Black Sea regions. Especially Middle and East Black Sea region soils are immature soil over the non-calcareous, low depth and mostly receive average of 1000 mm rain. Özyazıcı et al. (2013) stated that 45.62% of soils in Middle and Eastern Black Sea Region are strong acid to low acid character, 61.15% of the soil is low calcareous and 2.97% extractable magnesium content is low, and 25.59% is medium. Liming is often applied to increase the pH level in hazelnut orchard soils with low pH level. Anonymous (2012) reported that, 90% of fertilizers used in hazelnut agriculture is nitrogen, and 10% covers other types. Hazelnut orchards located in Black Sea region receive too much rain and 45% of them have low pH. Because especially liming and nitrogen fertilizers are overused, often encounters with lack of magnesium.. Güneş et al. (2002) stated that magnesium deficiencies reason is lowest amount of found in soil, but the amount of other cautions such as H^+, K^+, NH_4^+, Ca^{++} and Mn^+ were also the reason of magnesium deficiencies.

Balanced nutrition is one of the most important factors for increasing yield and improving quality of hazelnut. Kernel ratio, total oil and protein contents, shelled and kernel nut weight, healthy nut, empty and wrinkle nuts ratio are some important quality parameters in hazelnut. These quality parameters are significantly affected by fertilization and nutrient interaction. Magnesium has substantial effects on increasing the yield and quality in hazelnut agriculture through interaction of magnesium with photosynthesis, formation of chlorophyll, synthesize of carbohydrate, and interactions of other plant nutrient elements. The features of hazelnut orchards and methods of plant nutrition in Turkey increase the importance of fertilization with magnesium. The objectives of this study were to indicate the effects on yield and quality in hazelnut agriculture with magnesium fertilization, to determine plant nutrient interactions with magnesium fertilization, and to determine appropriate level of magnesium fertilizers in hazelnut cultivation.

MATERIALS AND METHODS

The study was conducted Tombul hazelnut orchards in Hazelnut Research Station between the years 2009 and 2011. The trial was designed as randomized complete block designs with five apply magnesium doses and three replications per treatment. The experiment was established the average of 20 to 25 years old Tombul hazelnut ocak with 4 branches based on transplanting system. Magnesium doses were applied with 0, 100, 150, 200 and 250 kg ha^{-1}. For the 500 hazelnut ocaks to be located in one hectare orchard given fertilizer doses each ocak were calculated as 0, 2040, 3060, 4080 and 5100 g $ocak^{-1}$ $MgSO_4.7H_2O$ and these doses were applied during 3 years. Also, according the results of soil analysis of the hazelnut orchards, other fertilizers were applied to each hazelnut ocak in required amounts. Texture analysis of the experiment were performed according to Soil Survey Staff (1951), pH and EC analysis according to U.S. Salinity Lab Staff (1954), $CaCO_3$ analysis according to Çağlar (1958), organic matter content according to Nelson and Sommers (1982), available phosphorus according to Bray and Kurtz (1945), available potassium according to Knudsen et. al. (1982) and extractable magnesium Anonymous (1982) were done. Required fertilizers were applied in the hazelnut orchards during 3 years. Cultural maintenance were made throughout the year such as pruning, weed control, disease and insect control, etc. Each hazelnut ocak was harvested separately in the first week of August depending on climate (Ayfer et al., 1986; Köksal, 2002). Harvested hazelnuts were separated from husk and dried. The dry shelled nut yield of each hazelnut ocak was found in per a hectare. For each nut samples, the following quality criteria's were analyzed for kernel ratio, shelled and kernel weights, percent healthy nuts, empty nuts, wrinkled nuts and shell thickness according to Ayfer et al. (1986) and Koksal (2002). Chemical compositions of hazelnut samples were analyzed for protein content according to Hartwitz (1970), and oil contents by Weende analysis methods according to Ayfer et al. (1986).

Statistical analyses were performed using analysis of variance in JMP statistical software. Results were expressed as means ± standart deviation (SD). Differences at $p<0.05$ were considered to be significant. All data obtained were the mean from the three years (Düzgüneş et al., 1983).

RESULTS AND DISCUSSION

In the trial hazelnut orchards, after fertilizing with $MgSO_4.7H_2O$ in increasing amounts, each year soil samples were taken and analyzed and these findings are represented in Tables 1 and 2. It was stated that, soil samples were clay loam, soil reaction changed between 5.91 and 6.19, EC changed between 0.08 and 0.17 $dS.m^{-1}$ and was not salinity problem, amount of calcareous changed between 0.05 and 0.21% and soil organic matter contents changed between 4.72 and 6.05%. Extractable magnesium content of orchard soil was found around 55.48 mg kg^{-1} in 0 kg ha^{-1} Mg (control). It was stated that Tombul hazelnut orchard soils, which magnesium fertilizer used, extractable magnesium

Table 1. The effects of magnesium fertilizing on some soil properties.

Treatments Mg (Kg ha^{-1})	Texture	pH (1:3)	EC (dS.m^{-1})	$CaCO_3$ (%)	Organic matter (%)
0	CL	6.02±0.157	0.100±0.020^{c}*	0.05±0.017^{d}	4.72±0.479^{b}
100	CL	6.03±0.582	0.080±0.026^{c}	0.14±0.020^{b}	5.51±0.877^{ab}
150	CL	6.19±0.346	0.083±0.015^{c}	0.10±0.017^{bc}	6.05±0.220^{a}
200	CL	6.09±0.355	0.140±0.020^{b}	0.21±0.032^{a}	5.12±0.642^{ab}
250	CL	5.91±0.382	0.170±0.020^{a}	0.09±0.017^{cd}	5.21±0.264^{ab}
LSD (5%)		0.7069	0.0376	0.0393	1.006

*Means (± standart deviation) followed by different letters in the same column are significantly different from each other at p < 0.05 level

Table 2. The effects of magnesium fertilizing on some soil properties, leaf and nuts magnesium contents.

Treatments Mg (Kg ha^{-1})	Available phosphorus (mg kg^{-1})	Available potassium (mg kg^{-1})	Extractable magnesium (mg kg^{-1})	Magnesium content at the leaf (%)	Magnesium content at the nut (%)
0	125.87±16.68a*	329.52±119.10	55.48±23.2^{b}	0.14±0.0115^{b}	0.15±0.0802
100	100.95±21.00ab	343.98±60.10	172.17±75.1^{ab}	0.18±0.0153^{a}	0.16±0.0802
150	101.01±13.78ab	416.86±208	260.30±142.9^{a}	0.19±0.0200^{a}	0.15±0.0854
200	88.24±3.45b	318.69±221	245.11±95.3^{ab}	0.21±0.0153^{a}	0.17±0.0624
250	84.28±24.00b	298.24±184	248.84±156.2^{ab}	0.19±0.0208^{a}	0.16±0.0702
LSD (5%)	31.502	308.526	119.454	0.0307	0.1385

*Means (± standart deviation) followed by different letters in the same column are significantly different from each other at p < 0.05 level.

content was “too low” and “low”. FAO (1990), soils are classified according to extractable magnesium content as < 50 ppm “too low”, 50-160 ppm “low”, 160-480 ppm “sufficient”, 480-1500 ppm “much” and > 1500 ppm “too much”. As indicated in the Table 2, in soil where magnesium fertilizing was not applied, amount of average available phosphorus was 125.87 mg kg^{-1}, and with magnesium fertilizing in increasing doses, this amount decreased to 84.28 mg kg^{-1}. It was found out that increasing amount of magnesium in soil decreased the amount of available phosphorus because of existing interactions. On the other hand, MgxP interactions prevented the increase rate of hazelnut yield. This interaction decreased kernel ratio and healthy nuts ratio, also increased the rate of empty and wrinkle hazelnuts. Kacar (1984) reported that Ca and Mg in soil increased the phosphor fixation regarding soil pH and as the rate of Mg and Ca increased in the soil, plants hardly used the phosphorus. It was seen that available potassium content was around 329.52 mg kg^{-1} where magnesium fertilizer was not applied in trial soils, and increased to 416.86 mg kg^{-1} with 150 kg ha^{-1} magnesium fertilization. However, when magnesium fertilizer doses were increased to 200 and 250 kg ha^{-1}, available potassium contents of trial soil decreased to 318.70 and 298.24 mg kg^{-1} respectively. Some amending agent increases the retention of Mg due to the increase of effective caution exchange capacity (resulting from the pH increase) and to an altered Ca/Mg ratio. The K may displace Mg from the exchange sites thereby increasing the content of the latter in the soil solution (Viade et al., 2011). The equilibrium of exchangeable cations in the soil is proportional to the activity ratio of K for divalent cations in soil solution (Quaggio et al., 2011). It was also determined that MgxK interactions in the soil due to magnesium fertilizing had negative effects on yield and quality properties. Aktaş (1994) reported that the action among plant nutrition elements known as antagonism was seen more among cations. The most known example of antagonism among plant nutrition elements was between magnesium and potassium, and K competed with Mg to be absorbed in soil ion absorption. Genç (1987) defined that potassium fertilizers increased the amount of hazelnut yields and decreased the amount of empty nuts. For the balanced fertilization in hazelnut cultivation, magnesium and potassium fertilizers should be applied so as to not cause antagonism.

Every year, after the annual fertilizing of the hazelnut orchards, the necessary cultural maintenances were made, and each hazelnut ocak was harvested separately in August and the amounts of dry shelled hazelnut yield were determined. The increasing magnesium fertilization effects on the yield are observed in Figure 1.

Approximately 1502.32 kg ha^{-1} dry shelled hazelnut

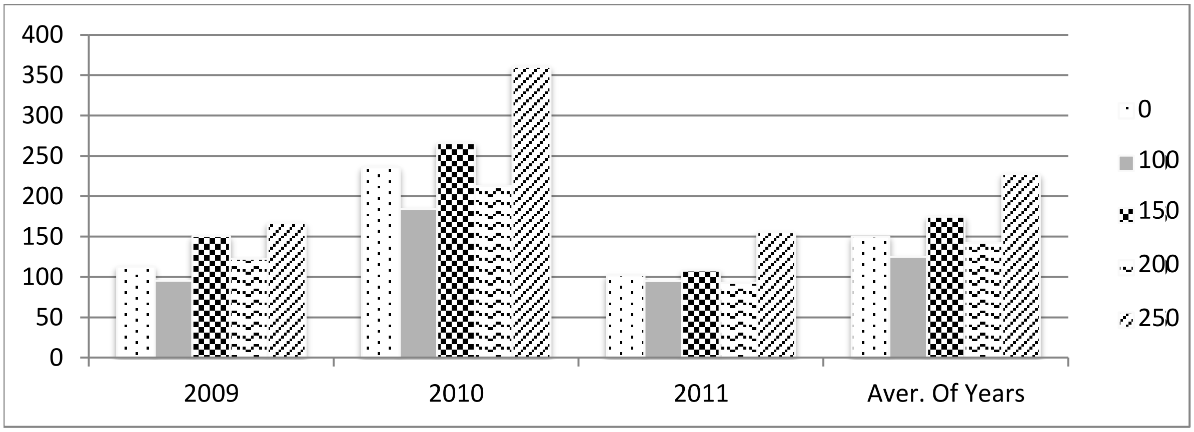

Figure 1. The effects of magnesium fertilizer on Tombul hazelnut yields (Kg.da^{-1}). LSD(5%): 145.499.

was obtained from 0 kg ha^{-1} application. The amounts of hazelnut yield increased with rising magnesium fertilizer doses and the highest amounts of yield to 2275.57 kg ha^{-1} was obtained from 250 kg ha^{-1} magnesium fertilization. Piroğlu and Genç (1970) stated that calcium ammonium nitrate and triple super phosphate fertilizers increases hazelnut yield as well as doing weed controls. Özenç and Çalışkan (2001) defined that the amounts of Tombul hazelnut yields were significantly increased with using mineral and organic fertilizers. Genç (1976) reported that the best fertilizing method to increase Tombul hazelnut productivity was as follows: 200 g ocak^{-1} nitrogen, 300 g ocak^{-1} phosphorus and 750 g ocak^{-1} potassium. When Figure 1 is examined, both magnesium fertilizers and years significantly affected on the yield of Tombul hazelnut. While the amounts of hazelnuts were higher in 2010 in total, these amounts had been lower in 2009 and 2011. This has stemmed from the periodicities that occur depending on the vegetal characteristics of the hazelnut. Çetiner (1976) has stated that the yield fluctuation in Tombul hazelnut is in low or middle level; Ayfer et. al., (1986) has stated that the yield fluctuation is named as periodicity and it is seen in little amounts in Tombul hazelnut variety.

The contents of magnesium nutrition element in leaf and nut samples and the amount of protein and total oil in nut samples were determined (Tables 2 and 3). The magnesium nutrition elements in leaf were changed from 0.14 to 0.21%. Magnesium contents of leaf were more increased to 0.21% with 200 kg ha^{-1} magnesium fertilization. Jones Jr. et. al. (1991) classified according to the amounts of magnesium contents in hazelnut leaf samples 0.15 to 0.25% as “little”, 0.25 to 0.50% as “enough” and >0.50% as “much” and Snare (2007) has classified <18% as “little”, 0.19 to 0.24% as “middle”, 0.25 to 0.50% as “enough”, 0.51 to 1.00% as “much” and >1.00% as “excessive”. According to the present study results, magnesium contents of hazelnut leaves were found little and middle. Quaggio et al. (2011) reported that increased leaf K caused decreases on leaf Ca, Mg and B. The magnesium nutrition elements in kernels were changed from 0.15 to 0.17%. Magnesium contents of kernels were more increased to 0.17% with 200 kg ha^{-1} magnesium fertilization. Magnesium fertilization was not a significant effect on the contents of magnesium in nuts. It was also detected that magnesium fertilization was no effect for the protein contents of the kernels and the protein contents were between 17.11 and 17.62%. Ayfer et al. (1986) classified the hazelnuts as <14.4% little, 14.5 to 17.4% middle and >17.5% much according to their protein ingredients. In a similar way, Köksal (2002) reported that the protein contents in Tombul hazelnut was meanly 17.51%; Şahin et.al. (1990) reported that the protein contents as between 13.26 to 18.70 %. The total oil contents in the hazelnut samples were found as meanly 59.47% with 0 kg ha^{-1} applications. The increasing magnesium fertilization was significantly increased the total oil contents of the hazelnut; and 61.86% total oil was found in 100 kg ha^{-1} magnesium fertilization, 62.35% in 150 kg ha^{-1} fertilization, 62.14% in 200 kg ha^{-1} fertilization, and 62.08% in 250 kg ha^{-1} magnesium fertilization. Köksal (2002) stated that the oil amount of Tombul hazelnut was meanly 64.60 and 93.08% of it was unsaturated oil and 6.92% of it was saturated oil; Koyuncu et al. (1997) stated that the oil ratio of Tombul hazelnut in Terme and Çarşamba region was meanly 57.16%. This study results showed that magnesium fertilization was significantly increased the amounts of total oil in hazelnuts.

The effects of magnesium fertilization on some quality properties of trial hazelnuts were determined like percentage of kernel, weights of shell and kernel nuts, healthy nuts, empty nuts, wrinkle nuts and shell thickness (Tables 3 and 4). It has been detected that the percentage of kernels were found to 50.25% with 0 kg ha^{-1} applications and this ratio was decreased to 48.54% with

Table 3. Some chemical and quality properties of Tombul hazelnut.

Treatments Mg (Kg ha^{-1})	Protein (%)	Total oil (%)	Kernel ratio (%)	In-shell weight (g)	Kernel weight (g)
0	17.36 ± 0.494	59.47 ± 1.880[b*]	50.25 ± 0.664[ab]	182.81 ± 15.48	90.54 ± 7.81
100	17.11 ± 1.010	61.86 ± 1.601[a]	50.75 ± 1.293[ab]	187.03 ± 20.30	92.18 ± 9.98
150	17.46 ± 0.407	62.35 ± 1.214[a]	51.76 ± 2.100[a]	200.69 ± 18.10	103.11 ± 6.80
200	17.62 ± 0.735	62.14 ± 0.601[a]	48.85 ± 1.361[b]	197.46 ± 7.18	99.39 ± 1.53
250	17.25 ± 0.225	62.08 ± 0.440[a]	48.54 ± 1.580[b]	195.39 ± 21.90	95.15 ± 2.85
LSD (5%)	1.1565	2.316	2.6815	31.6581	15.741

*Means (± standart deviation) followed by different letters in the same column are significantly different from each other at $p < 0.05$ level.

Table 4. Some quality properties of Tombul hazelnut.

Treatments Mg (Kg ha^{-1})	Healthy nuts (%)	Empty nuts (%)	Wrinkle nuts (%)	Shell thickness (mm)
0	87.89±4.19	7.17±3.69	3.39±2.17	1.28±0.0954[ab]
100	89.06±5.02	7.39±5.26	2.00±1.30	1.32±0.0709[ab]
150	91.94±4.35	4.06±2.43	2.22±0.95	1.34±0.0379[a]
200	87.56±1.59	7.17±2.49	2.89±2.22	1.31±0.0265[ab]
250	84.39±7.77	6.83±3.24	5.22±3.47	1.22±0.0529[b]
LSD (5%)	9.080	6.502	4.009	0.1123

*Means (± standard deviation) followed by different letters in the same column are significantly different from each other at $p < 0.05$ level.

increasing amounts of magnesium fertilization. Ayfer et al. (1986), Çalışkan (1995), Çalışkan and Özenç (2001), Köksal (2002) stated that the kernel value of Tombul hazelnut changed between 49.9 to 51.7%. Decreasing kernel percentage has a significant loss of yield and quality properties. According to the present results, magnesium fertilization in hazelnut cultivation has to be made carefully in order to prevent loss of kernel quality. The weights of shell and kernel in 0 kg ha^{-1} application were between 182.81 and 90.54 g; these weights increased to 200.69 and 103.11 g with 150 kg ha^{-1} magnesium fertilization and the applications of more magnesium fertilizer were a negative effect on the weights of shell and kernel nuts. Ayfer et al. (1986) and Köksal (2002) stated that 100 kernel weights were meanly 96 and 90 g. The percentage of healthy nuts in 0 Kg ha^{-1}application was 87.89%, this amounts increased to 91.94% with the 150 kg ha^{-1} magnesium fertilization; however, the application of magnesium fertilizer in more amounts were decreased to the ratio of healthy nuts to 84.39%. Bostan (1997) informed that the healthy nuts ratio in Tombul hazelnut was 73.26%; Okay et al. (1986) informed that the healthy nuts ratio were increased in maintained hazelnut orchards and also wrinkle nuts ratio were decreased. The empty nuts ratio decreased meanly until 4.06% in 150 kg ha^{-1} magnesium fertilization and in the other applications, the empty nuts ratio increased to approximately 7% were identified. It was detected that the wrinkle nuts amount of the hazelnuts were meanly to 3.39% with 0 kg ha^{-1} applications. The wrinkle nuts amount decreased until 2.00 and 2.22% with 100 kg ha^{-1} and 150 kg ha^{-1} magnesium fertilization applications; with the increasing 200 and 250 kg ha^{-1} applications, the amounts of wrinkle nut could increase again meanly until 2.89 and 5.22%. Ayfer et al. (1986) and Köksal (2002) classified the hazelnuts as <3% as very little, 3 to 10% as little, 11 to 19% as middle, 20 to 29% as much and >30% as excessive according to their wrinkle amounts; and <3% as very little, 4 to 6% as little, 7 to 10% as middle, 11 to 14% as much and >15% as excessive according to their empty nut amounts. The shell thicknesses were found to 1.22 mm to 1.34 mm in the experiment hazelnuts. When the findings about the characteristics of quality and nutrient interactions in soils evaluated together, 150 kg ha^{-1} up to a dose of magnesium fertilizer was found to be effective. The more increasing magnesium fertilizer doses negatively affected the quality properties of hazelnut.

Conclusion

The results presented here evidence significant relations to plant nutrient interactions in soil and nut quality properties on Turkish hazelnut cultivars. In this study was detected that the use of magnesium fertilizer in hazelnut agriculture was a significant increasing the yield and quality properties. However, because of the interactions of magnesium nutrition element in the soil, the fertilizers used should be done carefully adjustment. Magnesium, phosphorus and potassium contents of soil were increased up to 150 kg ha^{-1} magnesium fertilization. When increasing the amount of magnesium fertilizer, significant interactions occur in soil systems. Although the highest yield was attained with 250 kg ha^{-1} magnesium fertilizer application, some quality properties of hazelnut were negatively affected due to the plant nutrition element interactions occuring in the soil. As the results of this study, the negative effects of nutrient element interactions in the soils were not observed up to 150 kg ha^{-1} Mg; hazelnut yield, the total oil, kernel percentage, in-shell and kernel weights, percentage of healthy nuts were increased and empty and wrinkle nut amounts were decreased through suitable magnesium fertilizer applications. When the soil, hazelnut yield and nut quality properties are considered together, it has been detected that the use of 150 kilogram magnesium fertilization per hectare in Turkish hazelnut cultivars is ideal.

Conflict of Interest

The authors have not declared any conflict of interest.

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