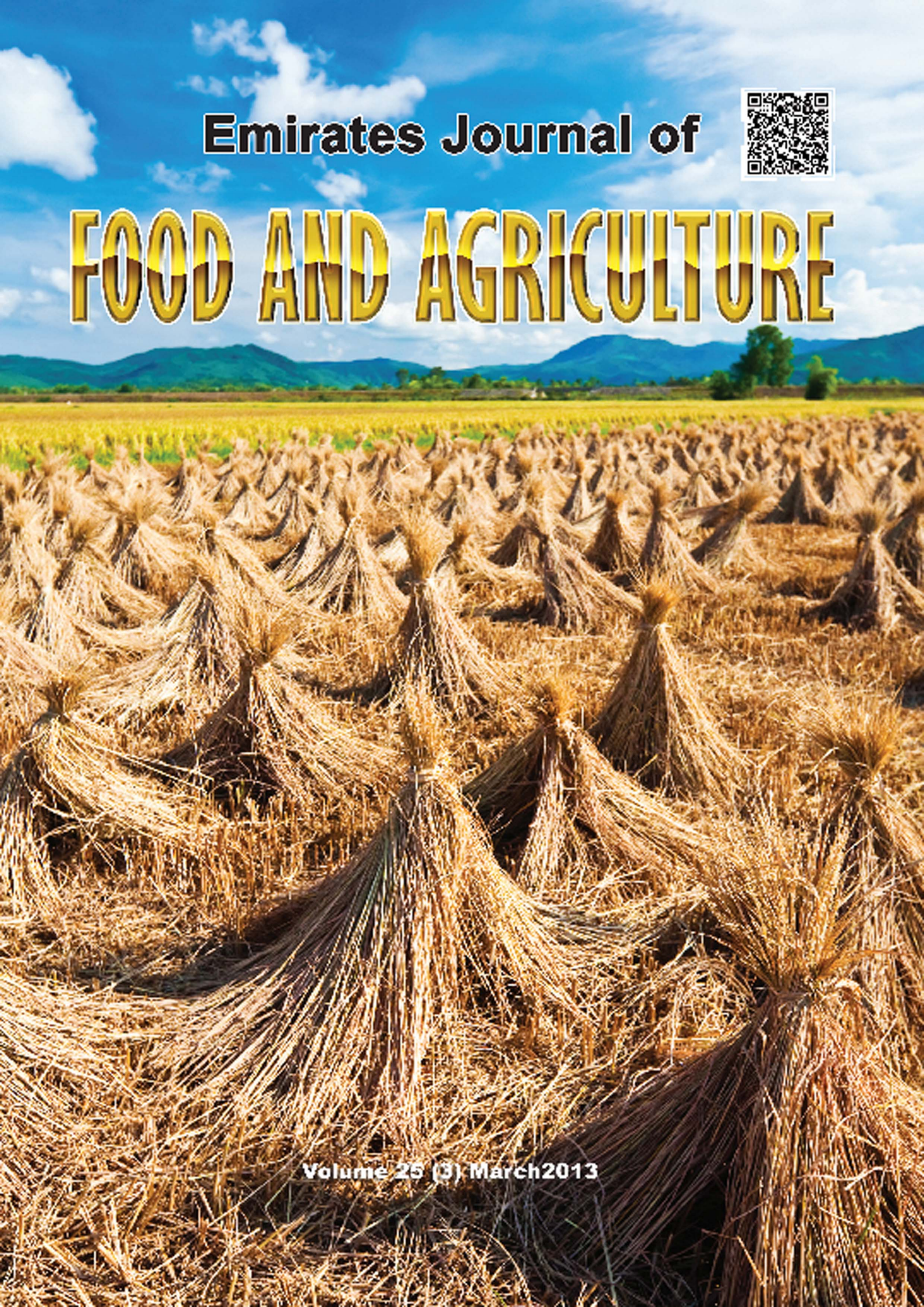
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NUTRITION AND FOOD SCIENCE

Consumer acceptability of chocolate chip cookies using applesauce as a fat (butter) substitute

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Abstract

Replacing fat in baked goods with fruit or vegetable base ingredients like applesauce would develop aid in the effort of overall reduction of fat intake and increase consumption of fruits and vegetables. The objective of this study was to determine the consumer acceptability of chocolate chip cookies prepared by replacing butter with applesauce. Three recipes were included: 1- Control: made according to Nestlé Toll House recipe. 2- Half replacement (HR): made by replacing 50% of butter with applesauce. 3- Full replacement (FR): made by replacing 100% of butter with applesauce. Nestlé Toll House recipe was employed in this study and Mott's applesauce was used for fat replacement. The 9 point hedonic rating scale system was used to evaluate the acceptability of the following sensory characteristics: appearance, texture, color, chewiness, sweetness, moistness, flavor, aftertaste and overall acceptability. Sensory evaluation was completed at North Carolina Agricultural and Technical State University on 35 food science students and employees. Our results showed that HR was very much acceptable, control was between very much acceptable and moderately acceptable, and FR was between moderately acceptable and neither acceptable nor unacceptable for all sensory characteristics. No significant ($P>0.05$) differences were reported in the acceptability of control and HR, whereas FR showed significantly ($P<0.05$) lower acceptability. Among all panelists ($n = 35$) 12, 16, and 7 panelists have chosen control, HR, and FR respectively as the most preferred and most worth buying cookie. In conclusion, applesauce could be an acceptable fat substitute in baked goods, thereby adding health benefits and nutritional value to baked products, and also contributing to the reduction of obesity.

Key words: Applesauce, Fat, Acceptability, Preferences, Sensory characteristic

Introduction

Overweight and obesity are epidemic in many parts of the world. Many countries have shown dramatic increases in overweight and obesity while no developed country is quite as heavy as the United States (Philipson and Posner, 2008). In the United States, 68.3% of adults older than 20 years and 48.1% of adolescents and children younger than 20 years are obese or overweight as of 2007-2008 (Shields et al., 2011). The United States has experienced a significant increase in obesity and overweight between 1980 and 2011 (CDC, 2011; Shields et al., 2011). These increases in obesity and overweight were associated with many chronic diseases including type 2diabetes, coronary heart disease, stroke, and high blood pressure (CDC, 2011). Much evidence suggests that the increases in obesity and overweight were related to the increases in fat and caloric intake (Cutler et al., 2003; Neuhouser et al., 2004; Philipson and Posner, 2008). Fat is an essential nutrient for human and one of the main food ingredients that play an important role in our food. Ultimately, a high fat diet may lead to an increased risk for numerous health problems such as obesity, cancer, cardiovascular disease, and type 2 diabetes (Cutler et al., 2003; Kafatos and Codrington, 2000; NIH, 2000; Philipson and Posner, 2008). To prevent negative health effects of fat intake, the National Institutes of Health has recommended that dietary fat be reduced from the current 35 – 45% of the total energy intake in most Western diets to below 25 – 30% (NIH, 2000). Therefore, during the last decade, consumers' demands in the United States and other western countries for low fat, reduced fat, and fat-free diets have increased substantially (CDC, 2011; Shields et al., 2011).

To improve the weight status and overall health, many researchers have focused on reducing

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the fat content in food products by replacing the fat with fruits or vegetables based ingredients. With this aim, oat dextrine (Shen et al., 2011), applesauce (Anding, 2008), avocado puree and oat rim (Wekwete and Navder, 2008), rice flour (Ali et al., 2011), pumpkin puree (Wang and Sullivan, 2010), okra gum (Romanchik-Cerpovicz et al., 2006), pureed eggplant (Doolittle, 2007), apricot kernel flour (Seker et al., 2010), hydrolyzed jicama starches (Amaya-Llano et al., 2008) and a composed of whey protein isolate, wheat starch, guar gum, xanthan gum or their blends (Kohrs et al., 2010) were tested in different food products as possible dietary fat substitutes. Replacing dietary fat with fruits and vegetables based ingredients will not only reduce the fat intake, although that will add additional health benefits to the produced food and contribute to increase the consumptions of fruits and vegetables. The consumption of fruits and vegetables in the United States are far short of the national target (CDC, 2011). The food industries in the United States are being encouraged to develop more enjoyable and acceptable products based on the use of fruits, vegetables, or their products. Baked food products are very much known to contain high fat, therefore, replacing fat in baked products with fruits or vegetables may contribute to healthier food. Fat is an important ingredient in baked products and contribute to the flavor, taste, texture, and final product acceptability (Jones, 2003; Colleen, 2007; Doolittle, 2007). There are different fat substitutes based on the content of carbohydrates, protein, or fat (Jones, 2003). Fat substitutes in baked products can be applesauce, prunes, eggplants, mashed banana, pureed fruit, non-fat buttermilk and non-fat yogurt (Jones, 2003; Colleen, 2007; Doolittle, 2007). Fat plays an important role in both taste and texture of baked products; therefore, choosing the appropriate fat substitute for a product or recipe is a critical step in replacing the fat. Applesauce is considered to be the best substitute for fat in fat-based baked goods such as quick breads, muffins and some cakes (Colleen, 2007;Anding, 2008). Applesauce is a puree made by cooking down peeled apples with water or apple cider to make unsweetened applesauce or adding sugar to be sweetened. Apples are not only high in pectin, apples are rich source of nutrients and phytochemicals (Gerhauser, 2008) and processing apples into applesauce has only a limited effect on the nutrients and phytochemicals in apples (Le Bourvellec et al., 2011). Apples also have many health benefits including: anticancer, antidiabetes and prevention of heart diseases (Gerhauser, 2008; Martineau et al., 2006). Apple polyphenols can also regulate fat metabolism and lower the level of LDL cholesterol (Nagasako-Akazome et al., 2007; Jensen et al., 2009). Replacing fat with applesauce in baked products will develop healthier food and may contribute to a reduction in overall caloric intake.

To the best of our knowledge, there has been no published research to evaluate the consumer acceptability of chocolate chip cookies prepared by replacing fat with applesauce. Therefore, the purpose of this study was to develop achocolate chip cookies recipes with applesauce as a fat substitute in order to create a healthier, lower fat, and lower caloric cookies, and then to evaluate the consumer acceptability of the new cookies based on the sensory characteristics and consumer preferences.

Materials and Methods

Nestlé Toll House recipe (Nestlé-toll-house, 2011) was used in this study as control recipe. Nestlé cookies are generally considered to be one of the most popular chocolate chip cookies in the United States. Three recipes were included: 1- Control: made according to the original Nestlé Toll House recipe, 2- Half replacement (HR): made by replacing 50% of the butter with applesauce, and 3- Full replacement (FR): made by replacing 100% of the butter with applesauce.

Ingredients for making chocolate chip cookies

Ingredients were purchased from local stores in Greensboro, NC. Table 1 shows the ingredients, brands, and amounts used in the control recipe. For HR recipe, 50% of the butter was replaced by 120 ml of Mott's applesauce (natural, no sugar added, nonchunky applesauce in a jar, Mott's Inc, Plano, TX) whereas other ingredients have remained the same. For FR recipe, several changes were made to control the moistness in the final product that include: Mott's applesauce was concentrated to reduce the moisture and 100% of the butter was replaced by 180 ml of concentrated Mott's applesauce, brown sugar was excluded and 300 g of granulated sugar was used as granulated sugar is free flowing and dry while brown sugar is moistly and sticky, one egg was added instead of two, and the other ingredients have remained the same. Concentrated applesauce was prepared by straining the applesauce for 15 min in order remove most of the liquid.

Table 1. Ingredients, brands and amounts used in the control and HR recipes to prepare the chocolate chip cookies.

Ingredient	Ingredient Brand	Amount
1. Flour	Pillsbury, unbleached, all purpose	280 g
2. Baking soda	Arm & Hammer	5 g
3. Salt	Morton	5 g
4. Butter, softened	Land Olakes butter	227 g
5. Granulated sugar	Domino Sugar	150 g
6. Packed brown sugar	C&H pure cane sugar, golden brown	150 g
7. Vanilla extract	Trader Joe's, pure vanilla flavor	5 ml
8. Large eggs	Egg- Land's best	2 (~ 50 g each)
9. Nestlé Semi sweet chocolate chip	Nestlé Semi sweet chocolate chip	340 g
10. Chopped nuts	Diamond walnut	100 g

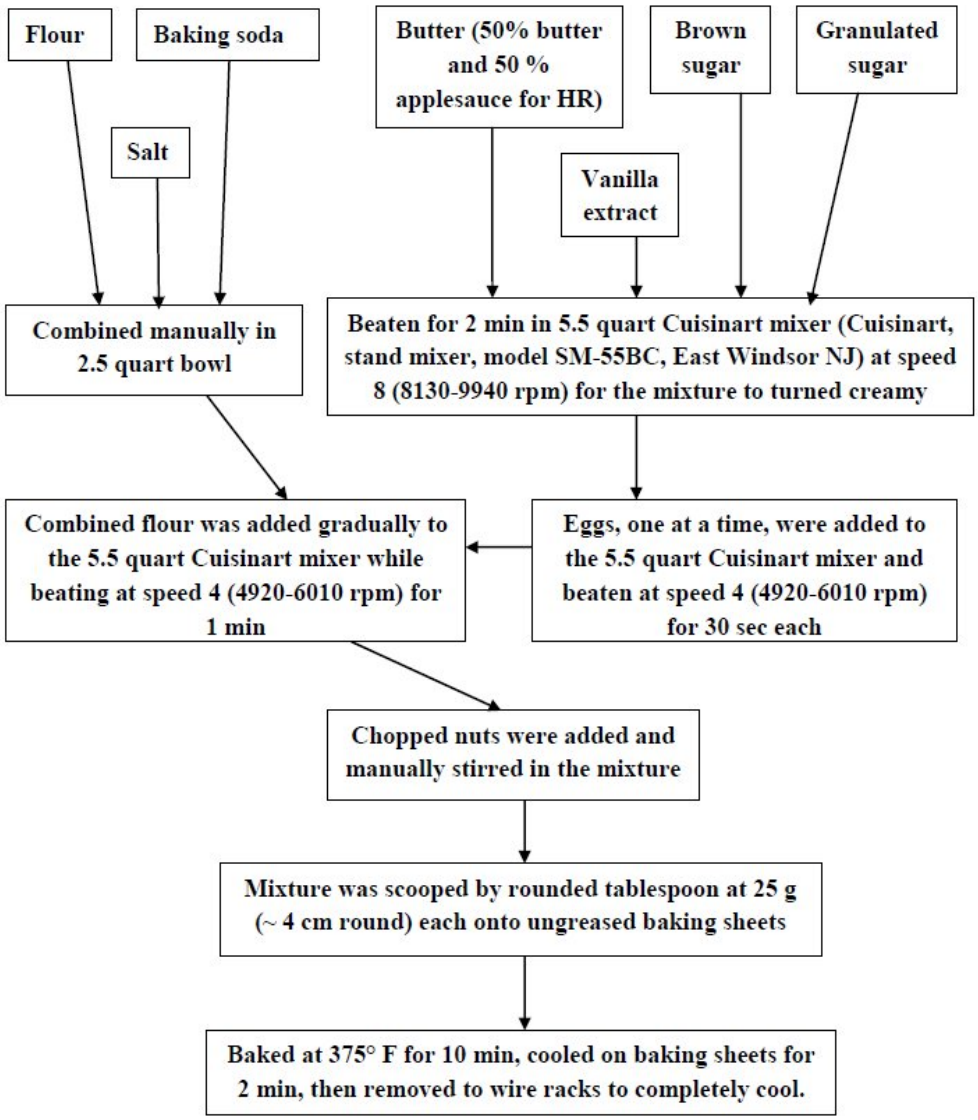

Figure 1. Flow chart diagram for preparing the control and HR recipes based on Nestlé Toll House recipe.

Preparation of chocolate chip cookies

Figure 1 shows the flow diagram for the control and HR recipes. The flow diagram was developed based on Nestlé Toll House recipe (Nestlé-toll-house, 2011). For FR recipe, in addition to the changes in ingredients, few additional changes were applied to the preparation method in order to control the moisture and improve the texture. After several initial trials, we found that replacing 100% of the butter with applesauce produced a more cake like product because of the high moisture content. In order to control the moisture level, the baking temperature was lowered to 340°F and the baking time was extended to 14 min. Also baking pans were greased to prevent stickiness of cookies, and all other steps have remained the same.

Sensory Evaluation

Chocolate chip cookies were prepared one day prior to sensory evaluation test. Sensory evaluation was designed based on consumer acceptability and preferences with regard to chocolate chip cookies. In this case, consumers evaluate baked products based on the physical appearance and color, texture and flavor, sweetness, moistness, and chewiness (Cauvain and Young, 2006). Panelists (n = 35) were food science students and staff members at North Carolina Agricultural and Technical State University. The 9 point hedonic rating scale system, with 9 for extremely acceptable and 1 for extremely unacceptable, was employed (Mc Williams, 2007). The sensory evaluation test has included the following characteristics: appearance, texture, color, chewiness, sweetness, moistness, flavor, aftertaste and overall acceptability. Preferences among the three cookies were evaluated by simply adding the following question at the end of the score card: Of the three products, which did you prefer the most and is most worth buying? The cookies were assigned the random numbers 515, 727, and 333 for control, HR, and FR respectively to reduce any bias. The cookies were placed on white plates separately with random arrangement and panelists were asked to make a random selection of one product at a time. The score card in Figure-1 was provided to each panelist prior to the sensory evaluation test and brief explanation was provided.

Statistical analysis

Statistical analysis of data was performed using SAS General Linear Model (GLM) program. The averages and standard deviations of scores for each characteristic were calculated and Duncan's Multiple Range Test was used to determine significant ($P<0.05$) differences. Percentage of acceptability was calculated based on the following equation:

$$\text{Percentage of acceptability} = \frac{\text{Total earned scores of the characteristic}}{\text{Total possible scores of the characteristic}} * 100\%$$

Results and Discussion

Table 2 shows the averages of scores for each characteristic from each cookie. Even though, HR has earned higher scores than control, no significant ($P>0.05$) differences were shown between control and HR except for moistness. Moistness of control was also significantly ($P<0.05$) the least acceptable among all control characteristics. The present of applesauce, as high moisture ingredient (58% water) (Mott's, 2011), raised the moisture content of the cookies and that was more acceptable among the panelists. However, only limited increase in moisture can be acceptable, extra moisture is not recommended for cookies and can cause cookie texture to become more like cake. Our preliminary study (not reported) showed that FR recipe prepared using regular applesauce and based on the same directions as the Nestlé Toll House recipe will make the final product more like cake instead of cookies. High moisture in fruits or vegetables based fat substitute plays an important role in determining the percentage of replacement between fat and substitute in order to avoid high moistness and maintain cookie texture. Therefore, changes in FR recipe were necessary to produce a chewy and crunchy cookie.

Table 2 also shows that FR averages of scores are significantly ($P<0.05$) lower than control and HR except for color. Color was the only characteristic with no significant ($P>0.05$) differences among the three cookies. The addition of applesauce was associated with the development of more brownish color cookie (see Figure 2), however, the three colors were fairly accepted with no significant ($P>0.05$) differences. This indicates that the addition of applesauce did not result in undesirable changes in color. The highest score among all FR cookie tested characteristics was shown on color (7.31 ± 1.92) the second highest FR score was shown on appearance (6.94 ± 2.12). Even though, average score of FR appearance was significantly ($P<0.05$) lower than control (7.94 ± 0.89) and HR (8.00 ± 1.08); FR appearance and color were moderately acceptable with no significant ($P>0.05$) differences among all FR tested characteristics. Average scores of FR appearance and color were significantly ($P<0.05$) higher than other FR tested characteristics. The finding here agree with others' findings namely that butter has limited contribution when it comes to color and appearance of baked cookies (Cauvain and

Young., 2006; Seker et al., 2010). The remaining FR tested characteristics were ranged between 5.20 (Neither acceptable nor unacceptable) and 6.14 (Slightly acceptable) and no characteristic was shown to be unacceptable.

Acceptability and Sensory Evaluation Test for Chocolate Chips Cookie made with Applesauce as fat (butter) substitute

Judge name:

Date:

There are three chocolate chip cookies labeled as 515, 727, and 333. You will need to evaluate one cookie at a time randomly and according to the following rating scale:

9 = Extremely acceptable	**4** = Slightly unacceptable
8 = Very much acceptable	**3** = Moderately unacceptable
7 = Moderately acceptable	**2** = Very much unacceptable
6 = Slightly acceptable	**1** = Extremely unacceptable
5 = Neither acceptable nor unacceptable	

Place a number in the appropriate box that best describes your response to the following attributes of the chocolate chip cookies:

Characteristics	**515**	**727**	**333**
Appearance			
Texture			
Color			
Chewiness			
Sweetness			
Moistness			
Flavor			
Aftertaste			
Overall			

Of the three products, which did you prefer the most and is most worth buying?
(Choose only one) _____

Figure 2. Sensory evaluation score card.

Table 2. Effect of fat replacement with applesauce on the consumer acceptability of the sensory characteristics of chocolate chip cookies.

	Chocolate chip cookies		
Characteristic	Control	HR	FR
Appearance	7.94±0.89 ^{aA}	8.00±1.08 ^{aA}	6.94±2.12 ^{bA}
Texture	7.34±1.27 ^{aAB}	7.86±0.83 ^{aA}	5.31±2.18 ^{bB}
Color	7.94±1.05 ^{aA}	8.14±0.76 ^{aA}	7.31±1.92 ^{aA}
Chewiness	7.26±1.33 ^{aAB}	8.11±0.72 ^{aA}	6.14±2.3 ^{bAB}
Sweetness	7.57±1.43 ^{aAB}	7.86±1.14 ^{aA}	5.51±2.67 ^{bB}
Moistness	6.90±1.37 ^{bB}	8.26±0.71 ^{aA}	5.80±2.58 ^{cAB}
Flavor	7.40±1.53 ^{aAB}	8.06±0.76 ^{aA}	5.20±2.89 ^{bB}
Aftertaste	7.50±1.19 ^{aAB}	7.91±1.33 ^{aA}	5.34±2.68 ^{bB}
Overall	7.30±1.03 ^{aAB}	7.94±0.87 ^{aA}	5.80±2.29 ^{bAB}

*Acceptability is based on the 9 point hedonic rating scale system, with 9 for extremely acceptable and 1 for extremely unacceptable.

*Averages with the same lower case letter in the same raw are not significantly different $P<0.05$.

*Averages with the same upper case letter in the same column are not significantly different $P<0.05$.

Figure 3 shows a top and side cut image of each cookie. The picture shows that replacing fat with applesauce resulted in slight changes in color, appearance, and texture. It can be seen that reducing the butter and increasing the applesauce content cause an increase in the brownish color, thereby reducing the shape uniformity, increasing the roughness of the cookie surface, and increasing the lightness and fluffiness of the texture. However, changes in color, appearance, and texture were insubstantial and had limited effect on the acceptability of the three cookies.

Figure 3. Picture of top and side cut of the three chocolate chip cookies. The picture shows slight differences in color, appearance, and texture with respect of replacing the fat with applesauce.

Figure 4 shows the percentages of acceptability of the tested sensory characteristics. Percentage of acceptability was defined as the total earned scores of the characteristic divided by total possible scores multiplied by 100%. Percentage of acceptability was used to determine the possible acceptance or rejection of each cookie. Rejection of one sensory characteristic was interpreted as rejection of the cookie as whole. Any percentage of acceptability lower than 50% was considered to be a rejection for the purpose of this study. HR cookie was the most accepted based on all sensory characteristics followed by control cookie then FR cookie. However, none of the sensory characteristics of the three cookies had shown rejection. Percentages of acceptability, for each cookie as a whole, were 81%, 89%, and 67% for control, HR, and FR respectively. Preference among cookies was also considered to determine the most preferred cookie and potential of commercial production. Preferences to cookie were determined by simply asking each panelist to choose one cookie as the most preferred and most worth buying. Among all panelists (n = 35) 12, 16, and 7 panelists have chosen control, HR, and FR respectively as the most preferred and most worth buying cookie. These results indicate that HR is the most acceptable cookie and FR is the least acceptable cookie. However, a fair percentage of acceptability (67%) was showed on FR and fair number (7) of panelists chose FR as the most preferred and most worth buying cookie. These results would suggest that FR could be acceptable among a moderate percentage of consumers and could thus be commercially produced.

Table 3 shows the nutritional information of the three cookies. Nutritional information was considered to determine the calories and fats content of the studied cookies. Nutritional information was calculated based on Nestlé Toll House chocolate chip cookie (Nestlé-toll-house, 2011) and Mott's applesauce (Mott's, 2011) nutritional facts. Percent daily values are based on 2,000 calories diet. Replacing butter with applesauce caused 13% and 24% of calories reduction in HR and FR respectively. Total fat, saturated fat, and cholesterol were also reduced significantly (see Table 3). Butter is the highest caloric ingredient in chocolate chip cookies followed by Nestle semi-sweet chocolate chips. Each chocolate chip cookie serving contains 37 calories from chocolate chips. Chocolate chips are an essential ingredient in chocolate chip cookies. Slight reduction in chocolate chips could be another option to reduce fat and calories in chocolate chip cookies. However, further sensory evaluation is necessary to determine the acceptable level of reduction in chocolate chips as that may affect the sensory characteristics.

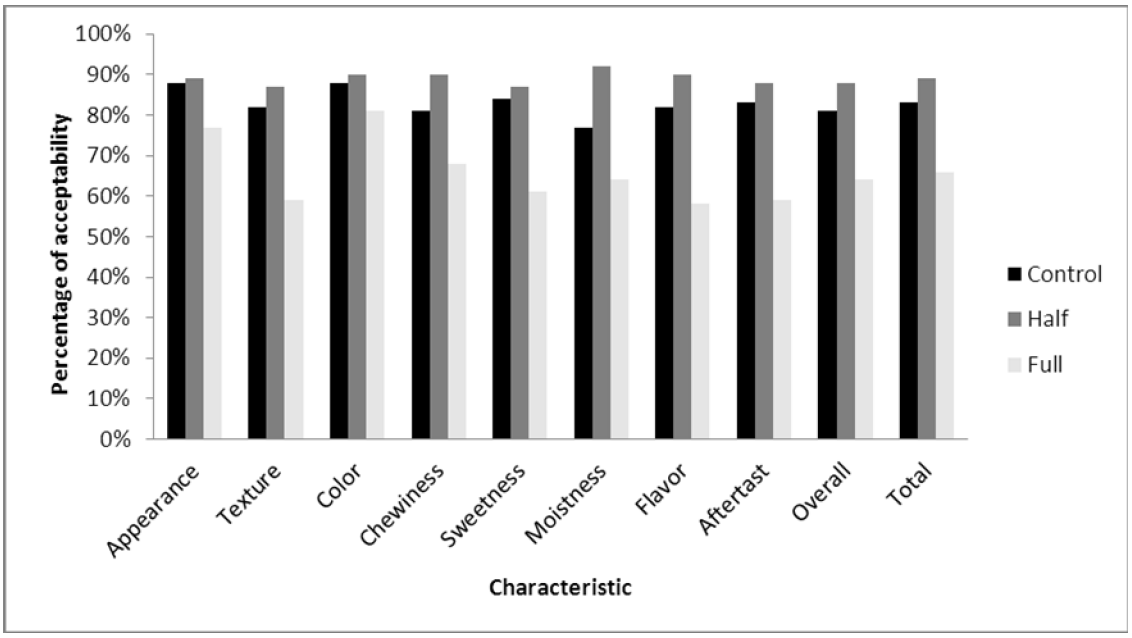

Figure 4. Effect of fat replacement with applesauce on the percentage of acceptability of the sensory characteristics of the tested chocolate chip cookies.

Table 3. Nutritional information of chocolate chip cookies calculated based on Nestlé Toll House chocolate chip cookies (Nestlé-toll-house, 2011) and Mott's applesauce (Mott's, 2011) nutritional facts.

Nutritional Information Per Serving (1 cookie) 21 g			
Content	Control	Half replacement	Full replacement
Calories	108	94	83
Calories from fat	55	41	27
Total Fat	6.2 g	4.6 g	3.0 g
Saturated fat	3.1g	2.1 g	1.0g
Cholesterol	15 mg	11 mg	3 mg
Total Carbohydrates	12.6g	12.8g	12.9g
Sugars	8.4g	8.6g	8.7g
Protein	1.4g	1.4g	1.3g

Apples and applesauce are notable for their high pectin content. Pectin in applesauce could be the main factor behind this successful replacement and sugar in applesauce could be another factor. Pectin works with gluten in the flour and helps with texture and structure just as fat does but in different technique. Fats are hydrophobic that can form a protective shields around the flour molecules and thus prevent the flour from combining with water to prevent the form of gluten (Jacob and Leelavathi, 2007). On the other hand, pectin is a gelling agent and specifically a polysaccharide. Pectin is hydrocolloid and does not actually protect the starch molecules (Lazaridoua et al., 2007). Pectin competes with the flour molecule**s** for water and sugar also attracts the water molecules**.** That means less water overall reaches the flour molecules, and because of this, the dough become less able to form gluten (Lazaridoua et al., 2007). On the other hand, applesauce has high water content which could support the forming of gluten. This means, too much applesauce in chocolate chip cookies will turn it to cake and too little applesauce will fail to achieve the goal benefits. As a general rule, determining the percentage between fat and fat's substitute is a major factor in developing the gluten and shaping the final product. The precise amount of fat varies depending on the recipe, and that is part of the issue with substituting applesauce in baked products. Other important issues to limit the amount of time in which gluten can develop are to mix the wet and dry ingredients at the last minute, and to blend them just briefly. The dough is not allowed to set and need to be baked immediately to avoid any possible gluten development. Thus when replacing fat in any baked products, it is important to understand the recipe, composition of substitute, mechanism of work, and type of fat in order to effectively choose the right fat substitute and right percent of substitution. Applesauce was recommended as the best fat substitute for oil-based baked products, like quick breads, muffins and some cakes (Colleen, 2007; Anding, 2008). In this study, applesauce was fully acceptable as a replacement of half of the butter and fairly acceptable as a full replacement for butter in chocolate chip cookies. Therefore, applesauce is an appropriate substitute of butter in chocolate chip cookies and could be used to replace the dietary fat in other baked products. For consumers who could accept slight changes in taste and texture (sweeter and softer), replacing 100% of fat in chocolate chip cookies with applesauce might be the right choice.

Conclusion

Overall, this study showed that applesauce can be a successful substitute of fat in chocolate chip cookies. Replacing butter with applesauce improves the nutritional value of chocolate chip cookies. Maintaining a proper balance between applesauce and fat is important for an acceptable cookie texture, because cookies tend to become softer in texture with an increase in applesauce. Our results further suggest that HR could be an alternative for the control in commercial production and FR might be accepted among fair number of consumers and could be commercially produced. The use of applesauce and other fruits or vegetables based ingredients to replace fat in baked products may also improve the consumption of fruits and vegetables. Applesauce thus merits more attention from the food industry as a successful fat substitute. Applesauce could also be used as a fat substitute in other baked products. Further work is needed to determine the storage conditions and shelf life of these cookies contained applesauce. In addition, further consumer sensory studies together with marketing research are needed to confirm the outcome of this study and to validate the potential of this new product in the U.S. market.

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NUTRITION AND FOOD SCIENCE

Value added products from nutri-cereals: Finger millet (*Eleusine coracana*)

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Abstract

Finger millet also known as ragi in India is one of the important cereals occupies highest area under cultivation among the small millets. Finger millet is comparable to rice with regard to protein (6-8%) and fat (1-2%) and is superior to rice and wheat with respect to mineral and micronutrient contents. It is a major source of dietary carbohydrates for a large section of society. Additionally ragi has enormous health benefits and also a good source of valuable micro-nutrients along with the major food components. In order to develop the value added food products based on ragi, that can able to enrich the nutritional value and also beneficial for good health is the current need for the wellbeing of the society. Food is consumed in combinations. The synergy between foods with other is vital not for taste and delight of eating but also for their high nutritional quality and health benefits. The modern trend for development of new food products aspires for complementary foods in order to fulfill the widening gap of food availability and nutritional security. This paper attempts to discuss the few value added food products incorporating ragi and simultaneously attempts to enlist and document the methodology and techniques in the interest of scientist and common mass.

Key words: Cereal grains, Finger millet, Nutritional properties of millets, Processing and value addition

Introduction

India is the leading producer of small millets namely, finger millet (*ragi*), kodo millet (*kodo*), foxtail millet (*kangni*), barnyard millet (*sawan*), proso millet (*cheema*) and little millet (*kutki*) (Majmdar et al., 2006). Annual planting area under them is around 2.5 million hectares; and nearly 1.5 million hectares is under finger millet comprising about 40-50% of crop's global area. During the last three decades, area under finger millet has declined but with the significant improvement in the productivity (1,500 kg/ha), its annual production is maintained at around 2.4 million tonnes. At present, small millets account for less than 1% of food grains produced in the world (ICAR, 2010). Their cultivation dates back to nearly 5000 years including India. Small millets form an important component of the traditional cropping systems and contribute significantly to the regional food and nutritional security and diversity in the national food basket. They are also important in areas of their production as dryland crops, as well as for hill agriculture. The small millet grains have longer storage life, and can be termed as famine reserve. The resilience exhibited by them may prove good for their adjustment to different eco-systems and make them potential crops for contingency plantings.

Cereals form a major portion of human diet and are an important source of starch and other dietary carbohydrates (dietary fibre), which play an important role in the energy requirement and nutrient intake of human. The millets are with higher fibre content, and their protein quality and mineral composition contribute significantly to nutritional security of a large section of population residing in the millet growing areas, considered to be the most disadvantaged groups (Desai et al., 2010). Millets are most recognized nutritionally for being a good source of minerals magnesium, manganese and phosphorus. Research has linked magnesium to a reduced risk for heart attack and phosphorus is important for the development of body tissue and energy metabolism. Millets are also rich in phytochemicals, including phytic acid (Shashi et al., 2007), which is believed to lower cholesterol, and phytate, which is associated with reduced cancer risk. Thus, millets are strategic in terms of their food, nutritional and livelihood security and their role in local agro-ecosystems

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(Joshi et al., 2008). The finger millet contains important amino acids viz., isoleucine (4.4 g), leucine (9.5 g), mthionine (3.1 g) and phenyl alanine (5.2 g) which are deficient in other starchy meals. Millets also contains B vitamins, especially niacin, B6 and folic acid calcium, iron, potassium, magnesium and zinc (Vachanth et al., 2010).

Finger millet is normally consumed in the form of flour-based foods such as *roti* (unleavened pancake), *mudde* (stiff porridge/dumpling) and *ambli* (thin porridge) and each of these foods have their characteristics features. The detail preparation methods have been reported by (Malleshi et al., 2007).

Finger millet or the *ragi* is usually used for preparation of flour, pudding, porridge and roti (Chaturvedi et al., 2008). With the changes in scenario of utilization pattern of processed products and awareness of the consumers about the health benefits, finger millet has gained importance because of its functional components, such as slowly digestible starch and resistant starch (Wadikar et al., 2007).

Traditionally *ragi* is processed either by malting or fermentation (Rao et al., 2001). Malting of finger millet improves its digestibility, sensory and nutritional quality as well as pronounced effect in the lowering the antinutrients. Malting characteristics of finger millet are superior to other millets and ranks next to barley malt (Pawar et al., 2007). There are various benefits of malting such as vitamin-C is elaborated, phosphorus availability is increased and lysine and tryptophan are synthesized (Desai et al., 2010). The malted and fermented *ragi* flour are extensively used in preparation of weaning food, instant mixes, beverages and pharmaceutical products (Rao et al., 2001).

Food uses of millets have, however, been confined only to traditional consumers; limited especially to areas of their cultivation, and still have remained underutilized. Processing them using traditional as well as contemporary methods for preparation of value added and convenience products would certainly diversify their food uses. Their exploitation for preparation of ready-to-use or ready-to-cook products would help in increasing the consumption of millets among non-millet consumers and thereby nutritional security. The present paper is an attempt to describe some basic information about finger millet, the processing requirement and some avenue for its value addition and food uses.

Experimental Details

In the present work two basic millets (*kodo* and *ragi*) of Chhattisgarh were considered for study. Samples of *kodo* and *ragi* were obtained from the local market. Samples were cleaned well before taking any experimental work. Value added products utilizing these grains were prepared keeping in mind the food habits in the niche area as well as broader areas adjoining the production catchments. In order to select the ratios of ingredients, several blends were prepared and processed into targeted foods. The foods were prepared using standard techniques described elsewhere. Sensory evaluation following 9-point hedonic scale for these foods was performed to select the best blending among the lots. To perform sensory evaluation, judges were drawn from the group of faculty and scholars of the university. The judges were trained and given pre-preparation tips for each of the foods and subjective measurement feelings. The blends rated highest among the lot were then selected for the final preparation. In order to determine the nutritional characteristics of these millets, standard methods (Sadasivam et al., 2005) described below were followed.

Moisture content

Moisture content was determined using oven following the method described in AOAC (2007).

Protein

Nitrogen content was determined following the Micro Kjaldhal method described in AOAC (2007) using Gerhardt Digestion and Distillation (model VAP-30), W Germany. Nitrogen was converted into protein by multiplying protein factor 6.25 (N x 6.25). Defatted sample was taken for the estimation.

Total Carbohydrate

Carbohydrate determination was done according to Hedge et al. (1962). It is hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose is dehydrated to form hydroxy methyl furfural which gives green colour with anthrone reagent (1mg/mL) at 630 nm. Glucose ($1x10^{-2}$ g/mL) is used as standard.

Fat

It is determined by solvent extraction method described in AOAC (2007) through Soxhlet Apparatus using petroleum ether as solvent.

Iron, Magnesium, Calcium

They were determined by AAS method following AOAC (2007).

Phosphorus

Phosphorus is determined by Colorimetric method at 660 nm following the procedure described in AOAC (2007).

Crude fibre

Crude fibre consists mainly of cellulose and lignin (97%) plus some minerals. It can be estimated by treatment of sample first with acid and subsequently with alkali. The loss in weight gives the crude fibre content (AOAC, 2007).

Results and Discussion

Nutritional characteristics

The nutritional characteristics of the two millets are presented in Table 1. Table also gives the comparison of properties of these millets with two major cereals of the world, wheat and rice. From the Table 1, it is clearly seen that the *ragi* millet is nutritionally superior compared to other three crops. *Ragi* millet contains fibre, more mineral and more vitamins, which are normally deficient in Indian diet and has eight times more calcium than other cereals. It is comparable to rice with regard to protein (6-8%) and fat (1-2%) and is superior to rice and wheat with respect to mineral and micronutrient contents. The calcium, phosphorus and iron contents of the millets were analyzed as these three minerals are of nutritional importance in the diets of population who consume millet as staple food. It is a major source of dietary carbohydrates for a large section of society belonging to millet growing areas. In addition to these, millets contain few other micro-nutrient compounds such as lignin, manganese, niacin etc. which are also important for human diet. However, their determination could not be done due to non-availability of the facilities.

Effect of millets on health

Magnesium from millets not only help to reduce the severity of asthma and migraine attacks, but also helps to reduce high blood pressure, diabetic heart disease, atherosclerosis and heart attack. Niacin is been used since ages to reduce high cholesterol levels in the body (Guigliano et al., 2011). Phosphorus from millets is an important mineral for energy production and is an essential component of Adenosine Triphosphate (ATP) – the energy store of the body (Shashi et al., 2007). It also forms an essential part of nervous system and cell membranes. A cooked cup of millet provides 26.4% daily value for magnesium and 24% daily value for phosphorus. Magnesium from millets also helps to relax blood vessels, enhances nutrient delivery by improving the blood flow and maintains the blood pressure and thus further protects the cardiovascular system. Lignin present in millets is converted to mammalian lignans by the healthy gut micro flora in our body which is thought to protect against breast cancer as well as heart diseases. The insoluble fiber from millets helps in gallstones prevention. A study proved that including insoluble fiber in diet lowers the risk of getting gallstones by 17% compared to women whose diet lack in fiber. This gallstones protection from fiber is dose related, with every 5g increase in insoluble fiber the risk drops by 10%. Millets helps to lower blood glucose levels and improves insulin response (Lakshmi et al., 2002). Finger millet is a humble grain with low glycemic index which makes it more suitable for diabetic patients **(**Pradhan et al., 2008). They maintain the sugar level of diabetic patient. Additionally, it is a rich source of calcium (344 mg/100g) and helps in supplementing the calcium in human body. Postmenopausal women with signs of cardiovascular disease like high blood pressure, increased cholesterol and obesity can benefit from eating whole grains especially millets by eating them 6 times a week. Whole grains like millet may have health promoting effects equal to or even in higher amount than fruits and vegetables and have a protective effect against insulin resistance, heart diseases, diabetes, ischemic stroke, obesity, breast cancer, childhood asthma and premature death (Cade et al., 2007).

Table 1. Composition of *kodo* and *ragi* millets (per 100 g edible portion, 12% moisture content).

S. No.	Particulars	*Kodo* millet	*Ragi* millet	Wheat*	Rice*
1.	Carbohydrate (g)	66.6	72.6	71.2	78.2
2.	Protein (g)	9.8	7.7	11.8	6.8
3.	Fat (g)	1.3	1.5	1.5	0.5
4.	Crude fibre (g)	9.0	3.6	12.9	5.2
5.	Ash (g)	2.6	2.7	1.5	0.6
6.	Calcium (mg)	27	344	41	10
7.	Phosphorus (mg)	188	250	306	160
8.	Iron (mg)	5.0	6.3	3.9	0.5
9.	Manganese (mg)	3.3	3.5	13.3	1.0
10.	Magnesium (mg)	228	130	120	32

* Source : FAO.1970, Rome, Italy, Nutritive value of Indian foods, 1998, NIN, Hyderabad, India

Value addition and value added products

In the foregoing paragraphs, some of the examples of value added products and possibilities of utilizing finger millet as one of the basic ingredients are discussed. Finger millet can be used in a variety of ways and is a great substitute for other grains such as rice and other starchy grains. These products are either in practice or have been demonstrated as avenue for enhanced consumption of finger millet. However, not much scientific studies have been carried out about their preparation and meaningful popularization on large scale.

(i) Multi-grain flour /Composite flour

The concept of multi-grain flour/composite flour is not new to the mankind. Mixing of two-three types of grains or grain and pulses has been in practice since long ago depending upon the availability of such commodities locally or the food habits, but in such cases, the understanding of nutritional security is not necessarily linked. Multi-grain flour by combining wheat and finger millet in the ratio of 7:3 (wheat:finger millet) is one of the simple semi-finished products suitable for making chapatti (roti), as no Indian meal is complete without Indian style bread or roti. In the proposed blend, though the gluten content is reduced significantly the making of chapatti while flattering is not affected. However, the colour of the chapatti turns to slightly dark. Fortification of finger millet in chapattis not only improves the taste but also helpful in controlling glucose levels in diabetic patients very efficiently (Kang et al., 2008). The bulkiness of the fibres and the slower digestion rate makes us feel fuller on, fewer calories and therefore may help to prevent from eating excess calories. Its high fiber content is further helpful to the individuals having the problem of constipation (Cade et al., 2007).

(ii) Papad

Addition of finger millet as one of basic ingredient to the tune of 15-20% (w/w) along with other essential ingredients such as black or green gram, rice and spices has become a tradition in millet growing areas of South India. According to a report, addition of finger millet up to 60% in papad is possible and practiced in some parts of Karnataka (Begum et al., 2007).

Papad from finger millet flour is also prepared in which it is used as base material mixed with spices and salt. Flour is first cooked in water till it is gelatinized and dough is prepared. Thin sheet from the dough is prepared by rolling it and cutting into desired shapes and sizes followed by drying of these papad pieces to desired moisture content of 7-8% (db). Since the pericarp is not separated out from the starch, it gives a little dark colour to the papad which again upon frying or roasting turns to lighter with good consumer acceptability.

(iii) Puffing or popping

Puffing or popping of cereals is an old practice of cooking grains since time immemorial to be used as snack or breakfast cereal like corn either plain or with some spices/salt/sweeteners. Popping or puffing of finger millet is one of the popular traditional methods and the popped millet and its flour is a RTE product with pleasing texture and appealing flavor. Popping improves the nutritional value by inactivating some of the antinutritional factors and thereby enhancing the protein and carbohydrate digestibility (Nirmala et al., 2000); it also enhances the appearance, colour, taste and aroma of the processed raw material. The flour can be used for different types of RTE food preparations depending upon the taste and likings. For puffing, the whole finger millet grain is conditioned by mixing additional water so as to reach its moisture content in the range of 18-20% and tempered for about 4-6 hours under shed. The conditioned grains are puffed by agitation on the hot sand surface maintained at about 230 - 250 ˚C for short time following HTST (high temperature and short time) process. During this process, the sugars present in the aleurone layer react with amino acids of the millet causing Millard reaction and as a result, a pleasant and highly desired aroma is developed. Further, during this process, the vapour pressure of the grain increases and the moisture present in the grain turns into steam; gelatinization of the starch takes places and explodes. Since during popping or puffing grains are dehydrated to the extremely low level of moisture content, nearly 3-5%, the shelf-life is enhanced. Now days modern air puffing machines have been developed which can be used for mass production of puffed or popped millet grains. In addition to this, there will be no risk of sticking sand particles with the product in machine popping or puffing.

(iv) Puffed finger millet mix

Puffed finger millet grains can be converted into powder by simple grinding which can further be enriched with additional ingredients. Various combination of ingredients can be taken and mix well, this nutritious mix so prepared forms RTE food. The selection and combination of the ingredients is done based on the requirement of the target groups like children, pregnant and lactating

mothers etc. The ingredients are selected in such a way that no further cooking requires and hygienically packed in suitable packaging materials. The following table give an example of such mix, similar other combination of ingredients can be selected which should be nutritious as well as acceptable to the target group. The mix contains higher amount of protein, energy, calcium and iron with higher bioavailability (Edam et al., 2001).

(v) Malting – Weaning food

Traditionally the millet malt is utilized for infant feeding purpose and also to prepare beverages either with milk of lukewarm water with the addition of sugar since pretty old times. Finger millet being good malting characteristics, its malting is popular in the area of cultivation particularly in Karnataka and part of Tamilnadu. Malting of finger millet improves its digestibility, sensory and nutritional quality as well as pronounced effect in lowering the antinutrients (Desai et al., 2010). Finger millet has some of the inherent qualities which make it superior compare to other cereals and also qualify for malting and preparation of malted foods. It is resistant to fungal infection and elaboration of alpha and beta amylase during germination and during roasting/kilning a desirable aroma as well as is developed which makes it an ideal grain for malt foods. In addition to these, finger millet is a good source of sulphur amino acids and calcium. An example of composite malt flour (malted weaning food) preparation combining finger millet, green gram and bengal gram is presented in the following process flow chart. This blend is nutritional in addition to rich source of protein and calcium.

In the above unit operations, the germination is an important unit operation which needs greater attention. During germination process, the hydrolytic enzymes bring changes in the endosperm. Some of the vitamins are also synthesized and the bioavailability of minerals increases. During soaking, the soak water is required to be changed once or twice to prevent the excessive growth of micro-organisms and also to make it free from CO_2 formed during soaking. During germination, it is essential to mix or turn the grains to provide good aeration to facilitate better germination. Germination period of about 48 hours is desired but in summer it can be reduced to 36 hours. To stop the germination process, the grains are dried either sun or mechanically drying. While drying it should be kept in mind that the drying temperature should not exceed 75˚C. Higher drying temperature may cause parboiling effect and hardening of the grains which may have adverse effect on milling and quality of the malt flour. The sprouted grains should be dried to a final moisture content of nearly 10-12% and subsequently the separation of roots and shoots is done which can be accomplished by various traditional and modern methods by giving a mild rubbing or abrasion action to the grain mass. These grains (malted) are then roasted uniformly at 70-80°C either by conventional toasting pan or heaters. Uniform heating and roasting helps in developing characteristic aroma and desirable quality of the product. The malt so obtained is pulverized to convert it into RTE form. The pulverization can be accomplished by any size reduction facilities suitable to convert into fine flour. The pulverized malt is then subjected to sieving through the fine sieve to separate the husk and fine malt flour is obtained.

The malted weaning food is mixed with powdered sugar, milk powder or whole milk along with flavouring agents to make as milk based beverage. This preparation is a good source of nutrition and suitable for all the age groups. This preparation is popularly known as '*ragi* malt' and can be used as health drink or energy drink. Now-a-days about 5% *ragi* malt is invariably blended with the energy food to improve its texture and mouth feel.

(vi) Noodles – Vermicelli

The changing food habits of children and teenaged groups have created a good market of noodles in India and abroad. The demand for millet noodles particularly the noodles made out of finger millet is growing due to awareness of its nutritional properties. Noodles are the pasta products also known as convenience foods prepared through cold extrusion system which become hard and brittle after drying. The cooking of these noodles is very convenient and requires few minutes. Noodles of different combinations are prepared such as noodles exclusively made of finger millet, finger millet and wheat in the ratio of 1:1 and finger millet blended with wheat and soy flour in the ratio of 5:4:1. In case of exclusive millet-based noodles, pre-treatment to the millet flour is given to facilitate extrusion and smooth texture which should retain while drying and cooking. Generally, in the preparation of noodles, wheat flour is invariably used as an important member of blend because the presence of wheat gluten has an added advantage which not only helps in easy extrusion but also gives a smooth and fissure free texture to the noodles. Several other combinations of blends can

be explored in the preparation of noodles keeping food values of ingredients and their availability in mind.

(vii) Extruded products

Extrusion technology is another novel way of transforming ingredients into value added products. Extruded products prepared from different grains are very popular now days among the all age groups and their demand is growing, one such example is 'Kurkure', very popular among children. The change in life-style is also bringing a drastic change in the food habits, and the extruded foods being RTE products have become a good choice as snack foods. All the cereals containing good amount of starch can be extruded after making flour and conditioning to required condition. Finger millet flour or grits exhibit good extrusion characteristics. Extrusion cooking has ability to gelatinize and cook the product to the fullest extent and enables its uses as a RTE food. In extrusion cooking, the combined effects of shear along with heat and pressure are mainly responsible for the modification of starch properties. The flour/grit with 16-18% moisture content has ability to extrude in the barrel temperature range of 100-120˚C well with good expansion index with crunchy, porous and smooth surface texture. Like other preparations, the finger millet flour can be blended with other legume ingredient flours in appropriate proportion with further fortification of minerals and vitamins to design a balanced nutritional extruded food. Alternatively, the extrudates can be pulverized and blended with calculated amount of other pre-prepared/cooked ingredients to prepare supplementary food mix for infant babies and lactating mothers etc. A further value addition of extrudates so prepared from finger millets can be done by coating with sweet or savory to attract children.

(viii) Bakery products

Incorporation of finger millet flour in the preparation of bakery products like biscuit, nan-khatai, muffins and bread has been attempted and efforts are being made to standardize the recipe and product quality. The use of millets in bakery products will not only superior in terms of fibre content, micronutrients but also create a good potential for millets to enter in the bakery world for series of value added products. In a recent study, attempts have been made to improve the nutritional quality of cakes with respect to the minerals and fibre content by supplementing with malted finger millet flour (Desai et al., 2010). In recent years, finger millet has received attention and efforts are under way to provide it to the consumers in convenient forms (Singh et al., 2012).

(IX) Fermented foods

Fermented foods like *Dosa* and *Idli* are popular in many parts of India. These are very common as breakfast foods and even as the evening meals in southern part of the country. Finger millet is widely used as one of the ingredient for these kinds of fermented foods. It not only improves the taste but at the same time enriches the food value in terms of protein, calcium and fibre. Sprouting of finger millet grain or the malted grains are also used for fermented foods depending on the taste and choice. *Ragi* flour is blended with the other base ingredients for fermented foods following other procedures.

(X) *Ragi* Soup

Ragi soup is prepared by mixing the *ragi* flour into water (one part *ragi* flour and 2.5 parts water). While mixing or stirring sufficient is taken so that that should not be lump formation and it should leave a smooth and thick body of mix. This mix is then heated for 15-17 minutes under medium heat or till it is cooked. During heating continuous stirring is needed to avoid any lump formation further. After cooking the mix is removed from the heat and mix with curd (half tea cup) and salt to taste. Leaving it for another 5 minutes it is ready to serve as warm. In case of cold serving, further cooling is required.

(XI) *Ragi* Pakora (finger millet fritters)

Cut onion lengthwise. Crush the garlic using a knife. Keep them aside. Mix *ragi* flour, crushed garlic, cumin seeds, red chili powder and salt to a bowl. Add 1/2 to 3/4 cup of water to the ingredients and make a more liquid like paste. Add the cut onion to the flour mix and coat it well with the mix. Heat oil in a pan. Once the oil is hot enough, add the flour coated onion to the oil and fry till it becomes crispy. Serve hot.

(XII) *Ragi* Vada

Chop onion and greens (Keerai). Keep aside. Take a vessel and mix all the ingredients except oil. Add required water and make a soft dough (like chapati dough), but slightly thinner than chapati dough. Heat oil in a pan. Take a small amount of dough, press that with help of fingers and drop that in hot oil. Fry till it turns into crispy or till the bubbles are almost stopped.

In addition to the above preparations, many other local preparations are in practice making use of finger millet depending upon the local habits and choice of the groups, some of them are common

across the regions but some typical products remain in the domain which needs to be popularized. Few modern products incorporating finger millet are now available in the market such as, *ragi* health drink (baby vita), foodles, multi-grain noodle, *ragi* biscuit, *ragi* vermicelli etc.

Finger millet is well comparable and even superior to many cereals in terms of mineral and micronutrient contents. Its major use as food has remained only in the area where it is cultivated and to the traditional preparations (Amadou et al., 2011). Finger millet has good potential of providing nutritional security to the consumers (Singh et al., 2012). Its consumption in urban area can be increased through its proper processing and value addition. With the advancement of post-harvest processing and value addition technologies, it has become possible to process and prepare value added products which are acceptable by both rural and urban consumers. This will not only help in increasing the profitability of its cultivators but will also help in providing income and employment opportunities in rural area.

Conclusion

In conclusion it can be stated that *kodo* and finger millets are staple food in different parts of India and abroad. Apart from, meeting the food requirements of the population of the cultivation catchment, they have sound nutritional and medicinal values. These properties however have not been focused to the residents of the non-millet growing regions. Though the consumption patterns of these millets are very specific and continue to remain regional specific, there popularization in the broader range is essential. Specific design of foods acceptable to the population of the region specific and group specific can help in promoting the millet consumption and thereby nutritional intake of the consumers significantly. This will also contribute to the food basket of the nation in addressing the food security.

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NUTRITION AND FOOD SCIENCE

Application of plastics and paper as food packaging materials – An overview

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Abstract

The role of plastics and paper as food packaging materials is reviewed with a brief outlook on the historical background of food packages in general. The inherent properties of these food packages that should be considered by food processors are also discussed. The current efforts in meeting the needs of consumers in ensuring food's quality with prolonged shelf life during storage and distribution were highlighted. This review article also reflects on the emerging trends in technology that address innovations on Modified atmosphere packaging (MAP), Active packaging (AP), Intelligent packaging (IP) and the use of anti-microbial agents to extend the shelf life of foods under storage and distribution conditions. The future of these packaging materials in the food industries and their impacts on the environment and the society at large will continue to receive attention.

Key words: Food, Packaging materials, Paper, Plastics, Antimicrobial

Introduction

Packaging materials provide a means to preserve, protect, merchandise, market and distribute foods. They play a significant role in how these products reach the consumers in a safe and wholesome form without compromising quality. The relationship between the food and contact with the packaging material continuously interact and contribute to changes that can occur over time in these products. It is therefore important that several factors are considered when choosing the right package for a particular food product. Generally, the packaging material may either be rigid or flexible. Rigid containers include glass and plastic bottles and jars, cans, pottery, wood boxes, drums, tins, plastic pots and tubes. They give physical protection to the food inside that is not provided by flexible packaging. Flexible packaging is a major group of materials that includes plastic films, papers, foil, some types of vegetable fibres and cloths that can be used to make wrappings, sacks and sealed or unsealed bags.

Both flexible and rigid packaging materials, alone or in combination with other preservation methods, have been developed to offer the necessary barrier, inactivation, and containment properties required for successful food packaging. The combination of rigid packaging materials made from metal, glass, or plastic with heat was shown to provide the most effective and widely used method for inactivating microorganisms (Cutter, 2002). However, there are other means by which plastic or paper packaging materials can inactivate microorganisms associated with foods, they include controlled atmosphere, vacuum, modified atmosphere, active, and edible packaging (Suppakul et al., 2003).

Since early man first used a variety of locally available natural containers to store and eat foods, significant developments in food packaging materials have provided the means to lower the growth of microbes as well as protect foods from external microbial contamination. Packaging materials were developed over the years to prevent the deterioration of foods by microbes resulting from exposure to air, moisture, or pH changes associated with the food or its surrounding atmosphere.

Food industries have to decide which packaging material will be more appropriate for their food product taking note of the advantages and disadvantages of their choice or perhaps what other attributes can be incorporated in the packaging material based on the end use properties of the food product. This review is mainly on the characteristics of plastics, paper as flexible

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packaging materials and their roles in food quality and safety.

Historical background

The earliest forms of packaging materials were leaves, hollowed-out tree limbs, grounds, skins, reed baskets and earthenware vessels as containers. As civilization developed, more complex containers were developed to meet specific needs. Large ceramic vessels, amphoras were used from 1500 BC to 500 AD to ship wine and other products commercially throughout the Mediterranean. The most large-scale use was to serve the ancient Greek and Roman empires. Although their form is much different from our current packages, the shape and design were clearly the result of the same reasoning that we use to design successful packaging today. They were designed to be economical, to produce and ship. The unusual shapes, and especially the pointed base, facilitated handling, storage, transport and use in logistical systems that were very differently shaped from those that we use today (Twede, 2002).

Glass-making began in 7000 B.C. as an offshoot of pottery, and was first industrialized in Egypt in 1500 B.C. Glass is made from base materials (limestone, soda, sand and silica), which were in plentiful supply, all ingredients were simply melted together and molded while hot. Although the mixing process and the ingredients have changed very little, the molding techniques have progressed dramatically. Paper (from stems of papyrus in ancient Egypt) is the oldest form of what is referred to as "flexible packaging". It was reported that sheets of treated mulberry bark were used as a flexible packaging material by the Chinese to wrap foods as early as the First or Second century B.C and during the next fifteen hundred years, the paper-making technique was refined and transported to the Middle East, then Europe and finally into the United Kingdom (Welt, 2005).

The use of metal containers as packaging materials started from ancient boxes and cups, made from silver and gold, which were too valuable for common use. Cheaper metals, stronger alloys, thinner gauges and coatings were eventually developed and mass produced. After metal cans were invented and progressively improved, it was necessary to find a way to open them. Until 1866, the only method was by hammer and chisel. It was during this period that the keywind metal tear-strip was developed and after nine years in 1875, the can opener was invented. The can opener remained for more than 100 years, the most efficient method of retrieving the contents from metal cans. In the 1950s, the pop top/tear tab can lid appeared and now tear tapes that open and reseal are popular (Hook and Heimlich, 2011).

Plastic is the youngest in comparison with other packaging materials. It was discovered in the 19th century, most plastics were reserved for military and wartime use. Styrene was first distilled from a balsam tree in 1831. But the early products were brittle and shattered easily. Germany refined the process in 1933 and by the 1950s foam was available worldwide. Insulation and cushioning materials as well as foam boxes, cups and meat trays for the food industry became popular. Vinyl chloride was discovered in 1835 and provided the opportunity for the further development of rubber chemistry. For packaging, molded deodorant squeeze bottles were introduced in 1947 and in 1958; heat shrinkable films were developed from blending styrene with synthetic rubber. Cellulose acetate was first derived from wood pulp in 1900 and developed for photographic uses in 1909.

DuPont manufactured cellophane in New York in 1924 but was not commercially used for packaging until the late 1950s and early 1960s. In 1933, films protected submarine telephone cables and later were important for World War II radar cables and drug tablet packaging. After the war, the new plastics that had been developed entered the consumer mainstream in a flood and ‘Tupperware’ polyethylene food containers with air tight seal entered the market in 1946 (Plastics Make It Possible report, 2010).

There were new manufacturing processes developed using various methods such as forming, molding, casting, and extrusion to churn out plastic products in vast quantities (Packaging Today report, 2012). Other cellophanes and transparent films have been refined as outer wrappings that maintain their shape when folded. Originally clear, such films can now be made opaque, coloured or embossed with patterns. The polyethylene terephthalate (PETE) container became available during the last two decades with its use for beverages entering the market in 1977. By 1980, foods and other hot-fill products such as jams could also be packaged in PETE. In 1986, aluminium trays were replaced by plastic, microwavable trays. Metallocene catalysed polyolefins was introduced in 1996 to reduce food waste. In 2000 polylactic acid from corn entered the packaging market signalling the return of bio based plastic (Plastics Make It Possible Report, 2010).

Commonly available food packaging materials

The most common food packaging materials are glass, wood, metal, plastics, paper and other flexible packages such as coatings and adhesives. Each of these packages offers unique advantages and disadvantages that have to be critically considered in making the right choice by the food processor.

Plastic materials are made up of large, organic (carbon-containing) molecules that can be formed into a variety of useful products, they are fluid, moldable, heat sealable, easy to print, and can be integrated into production processes where the package is formed, filled, and sealed in the same production line (Marsh and Bugusu, 2007). The major disadvantage of plastics is their variable permeability to light, gases, vapours, and low molecular weight molecules. Structural polymers such as polyethylene and polypropylene provide mechanical properties at low cost, while barrier polymers such as polyvinyldene chloride and ethylene vinyl alcohol provide protection against transfer of gases, flavours and odours through the package. Tie resins, co-extrudable adhesive resins, bond the structural and barrier resins together.

The use of plastics in packaging has increased worldwide with an estimate at 280 metric tonnes (Paine and Paine, 2012). The packaging industry is the largest user of plastics; more than 90% of flexible packaging is made of plastics, compared to only 17% of rigid packaging. Barrier resins are generally being employed for plastic containers by modifications to improve product protection and make them more cost effective.

Recyclable and Recycled Plastics

There are more than thirty different plastics in packaging; the most common are polyolefins, polyvinyls and polyesters. There are possibilities that chemical contaminants in plastic packaging intended for recycling may remain in the recycled material and could migrate into the food. Other aspects of plastics recycling, such as microbial contamination and structural integrity of the recycled plastic, are also important considerations for the safe use of recycled plastics for food-contact applications.

Plastic recyclers must be able to demonstrate that contaminant levels in the reformed plastic have been reduced to sufficiently low levels to ensure that the resulting packaging is of purity suitable for its intended use. The production of a polymer with the desired qualities will require additional antioxidants, processing aids, or other adjuvants that may need to be added to the recycled polymer (CFSAN, 2006).

As petroleum reserves become more limited, new varieties of plastics are likely to increasingly be made from renewable biomass. These will contribute to the already extensive array of mechanical and aesthetic performance properties that plastics are well known for. The utilization of fossil fuels in the manufacture of plastics accounts for about 7% of worldwide oil and gas (Okada, 2002). These resources will arguably be depleted within the next one hundred years, and the peak in global oil production as estimated by some will occur within the next few decades. The plastic industry will be faced with real issues associated with the use of an essentially non-renewable feedstock for the majority of their products and there is an urgent need to develop new synthetic routes to polymeric materials using renewable resources (Williams and Hillmyer, 2008). Current packaging designs are beginning to incorporate recyclable and recycled plastics but the search for reuse functions continues. There are several factors that play into the economic assessment of recycling, including costs for collection, separation, cleaning or reprocessing, and transportation (energy).

Table 1. Common abbreviations for different plastic films and coating materials.

Abbreviation	Full form
PE	Polyethylene
PP	Polypropylene
PET or PETE	Polyethylene terephtalate
PEN	Polyethylene naphthalene dicarboxylate
PC	Polycarbonate
EVA	Ethylene vinyl acetate
PA	Polyamide
PVC	Polyvinylchloride
PVdC	Polyvinylidene chloride
PS	Polystyrene
SB	Styrene butadiene
ABS	Acrylonitrile butadiene styrene
EVOH	Ethylene vinyl alcohol
TPX	Polymethyl pentene
HNP	High nitrile polymers
PVA	Polyvinyl alcohol
HMT	Hexamethylene-tetramine

Recycling diverts materials from the waste stream to material recovery. It is different from reuse, which involves using a returned product in its original form, recycling involves reprocessing material into new products. The recycling program entails collection, sorting, processing, manufacturing, and sale of recycled materials and

products. It was shown that in order to make recycling economically feasible, recycled products and materials must have a market and the rates of recycling for plastics is on the rise in the United States of America (EPA, 2006).

Commonly used plastic films and their abbreviations are shown in Table 1. There are several plastic packaging materials for foods as shown in Figure 1. All thermoplastics are recyclable i.e they can be melted and re-used as raw materials for the production of new products. The recycling process requires separation by resin type as identified by the American Plastics Council and shown in Table 4.

PVC and PS are difficult to recycle. There are concerns that plasticizers such as adipates in PVC may leach to foods and incineration is a problem because of its chlorine. PS in an expanded form may be used for non-food packaging and cushioning and can then be recycled or incinerated (Marsh and Bugusu, 2007).

The above six commonly recycled plastic resin find wide applications in the following:

- PET: beverage bottles, mouthwash bottles, boil in bag pouches
- HDPE: milk jugs, trash bags, detergent bottles
- PVC: cooking oil bottles, packaging around meat
- LDPE: grocery bags, food wrap, bread bags
- PP: yoghurt containers, shampoo bottles, straws, margarine tubs, diapers
- PS: hot beverage cups, take-home boxes, egg cartons, meat trays

Apart from plastics and plastic products, other flexible packages include:

Paper products - Paper like webs of mixed cellulose and plastics, papers made from plastics, bonded fibre plastics, cloths and scrims, spun bonded fabrics, regenerated cellulose films, aluminium and steel foils.

Coatings and adhesives - Cellulose esters, cellulose ethers, rubber hydrochloride, chlorinated rubbers, chlorinated polyolefins, natural and synthetic bitumens and asphalts, natural and synthetic resins, adhesives of all types, prime, key, bond or sub-coats, latex bond mineral coatings, deposited metal layers.

Figure 1. Some examples of plastic packaging materials.

Table 2. Resin identification codes for plastics.

Resin	Code	Amount generated (thousand tonnes)	Amount recycled (thousand tonnes)
Polyethylene terephthalate	1	2860	540
High-density polyethylene	2	5890	520
Polyvinyl chloride	3	1640	-
Low-density polyethylene	4	6450	190[a]
Polypropylene	5	4000	10
Polystyrene	6	2590	-
Other resins	7	5480	390

Adapted from American Plastics Council, 2006b; a includes linear low density polyethylene

Paper and paperboards

Paper and paperboard are sheet materials made from an interlaced network of cellulose fibers derived from wood by using sulfate and sulfite. The fibers are then pulped and/or bleached and treated with chemicals such as slimicides and strengthening agents to produce the paper product. Paper and paperboards are commonly used in corrugated boxes, milk cartons, folding cartons, bags and sacks, and wrapping paper.

Paper and paperboards provides mechanical strength, they are biodegradable and have good printability. Coatings such as waxes or polymeric materials can be used to improve their poor barrier properties. Apart from their poor barrier properties to oxygen, carbondioxide and water vapour other drawbacks include their being opaque, porous and not heat sealable (FCIS report, 2011).

A few examples of paper packages for foods are shown in Figure 2 below. Polyethylene terephthalate (PET) is a desirable packaging material. It combines good barrier properties, clarity, impact resistance, and high speed processes have made PET containers a choice for carbonated beverage containers, dressings, edible oils, peanut butter and many other products. The many different types of paper used in food packaging can be categorized as follows (Kirwan, 2003, Marsh and Bugusu, 2007):

Kraft paper—produced by a sulfate treatment process, kraft paper is available in several forms: natural brown, unbleached, heavy duty, and bleached white. The natural kraft is the strongest of all paper and is commonly used for bags and wrapping. It is also used to package flour, sugar, and dried fruits and vegetables.

Sulfite paper—lighter and weaker than kraft paper, sulfite paper is glazed to improve its appearance and to increase its wet strength and oil resistance. It can be coated for higher print quality and is also used in laminates with plastic or foil. It is used to make small bags or wrappers for packaging biscuits and confectionary.

Greaseproof paper—greaseproof paper is made through a process known as beating, in which the cellulose fibers undergo a longer than normal hydration period that causes the fibers to break up and become gelatinous. These fine fibers then pack densely to provide a surface that is resistant to oils but not wet agents. Greaseproof paper is used to wrap snack foods, cookies, candy bars, and other oily foods, a use that is being replaced by plastic films.

Glassine—glassine is greaseproof paper taken to an extreme (further hydration) to produce a very dense sheet with a highly smooth and glossy finish. It is used as a liner for biscuits, cooking fats, fast foods, and baked goods.

Figure 2. Some examples of paper food packages, polyethylene (PE) added to increase stiffness and strength.

Parchment paper—parchment paper is made from acid-treated pulp (passed through a sulfuric acid bath). The acid modifies the cellulose to make it smoother and impervious to water and oil, which adds some wet strength. It does not provide a good barrier to air and moisture, is not heat sealable, and is used to package fats such as butter and lard.

Paper laminates are coated or uncoated papers based on kraft and sulfite pulp. They can be laminated with plastic or aluminum to improve various properties. For example, paper can be laminated with polyethylene to make it heat sealable and to improve gas and moisture barrier properties. Laminated paper is used to package dried products such as soups, herbs, and spices (Marsh and Bugusu, 2007).

Paperboards on the other hand are thicker than paper with a higher weight per unit area and often made in multiple layers. They are commonly used to make containers for shipping—such as boxes, cartons, and trays—they are seldom used for direct food contact. The various types of paperboard are as follows (Soroka, 1999; Marsh and Bugusu, 2007).

White board—made from several thin layers of bleached chemical pulp, white board is typically used as the inner layer of a carton. White board may be coated with wax or laminated with polyethylene for heat sealability.

Solid board—possessing strength and durability, solid board has multiple layers of bleached sulfate board. When laminated with polyethylene, it is used to create liquid cartons (known as milk board). Solid board can also use to package fruit juices and soft drinks.

Chipboard—chipboard is made from recycled paper and often contains blemishes and impurities from the original paper, which makes it unsuitable for direct contact with food, printing, and folding. It is often lined with white board to improve both appearance and strength. The least expensive form of paperboard, chipboard is used to make the outer layers of cartons for foods such as tea and cereals.

Fiberboard—Fiberboard can either be solid or corrugated. The solid type has an inner white board layer and outer kraft layer and provides good protection against impact and compression. When laminated with plastics or aluminum, solid fiberboard can improve barrier properties and is used to package dry products such as coffee and milk powder. The corrugated type, also known as corrugated board, is made with two layers of kraft paper with a central corrugating (or fluting) material. Fiberboard's resistance to impact abrasion and crushing damage makes it widely used for shipping bulk food and case packing of retail food products.

The packaging material as a barrier to gases and vapours

The food manufacturer incorporates food packaging materials that will act as a barrier to gases and water vapour. Oxygen and water vapour are major concerns in food packaging in relation to shelf life. The presence of oxygen in a packaged food is often a key factor that limits the shelf life of a product. Oxidation can cause changes in flavour, colour, and odour, as well as destroy nutrients and facilitate the growth of aerobic bacteria, moulds, and insects. Therefore, the removal of oxygen from the package headspace and from the solution in liquid foods and beverages has long been a target of the food-packaging scientists. The deterioration in quality of oxygen sensitive products can be minimized by oxygen scavengers that remove the residual oxygen after packing. Existing oxygen scavenging technologies are based on oxidation of one or more of the following substances: iron powder, ascorbic acid, photo-sensitive dyes, enzymes (such as glucose oxidase and ethanol oxidase), unsaturated fatty acids (such as oleic, linoleic and linolenic acids), rice extract, or immobilized yeast on a solid substrate (Floros et al., 1997). These materials are normally contained in a sachet. Oxygen scavenging is an effective way to prevent the growth of aerobic bacteria and moulds in dairy and bakery products. There are more details on oxygen scavenging from other reviews (Miltz et al., 1995; Miltz and Perry 2000; Floros et al., 1997; Vermeiren et al., 1999).

The barrier properties and capacity to protect foods depends largely on the permeability of the packaging material to gases and vapours. It was shown that the protection of foodstuffs may be achieved with a single layer of polymer or the use of multi-layered films including different polymers, coating and metal foils (Robertson, 2006). The moisture vapour transmission rate (MVTR) of single – ply films is an important criterion in the prevention of moisture and subsequent reduction of microbial growth that can lead to food spoilage. Hirsch (1991) investigated the MVTR for several single-ply packaging materials kept at 40ºC and 90% relative humidity. It was observed that Polyvinyldene chloride (PVDC) with a very low MVTR of 0.9 g/25µ/m^2/24h was better at preventing moisture when compared to Barex 210 with a high MVTR of 94.6 g/25µ/m^2/24h.

As with all food products, it is necessary to integrate a HACCP-based program to assure quality throughout the packaging operation. In addition to packaging improvements, other novel technologies that can be employed include the development of detectors for oxygen levels, bacterial toxins, and microbial growth, or the integration of time-temperature indicators for detection of improper handling or storage (Cutter, 2002). The main criterion to extending shelf life is to find a material that will balance the oxygen and carbon dioxide permeability and water vapour in a package.

Recent innovations on food packaging agents

Modified Atmosphere Packaging (MAP) is a form of packaging that involves the removal of air from the pack and its replacement with a single gas or a mixture of gases (Blakistone, 1999). Active packaging has been defined as a form of modified atmosphere packaging, which 'changes the condition of the packed food to extend shelf-life or to improve safety or sensory properties, while maintaining the quality of packaged food'. This can be achieved by the incorporation of certain additives into the packaging film or within a packaging container to modify the headspace atmosphere and to extend the product's shelf life. Intelligent packaging system monitors the condition of packed foods to give information about the quality of the packaged food during transportation and storage (Ahvenainen, 2003).

In recent years, antimicrobial packaging has attracted much attention from the food industry because of the increase in consumer demand for minimally processed, preservative-free products. As a result of this demand, the preservative agents must be applied to packaging in such a way that only low levels of preservatives comes into contact with the food (Cha and Chinnan, 2004). The use of appropriate film or coatings can impart anti-microbial (AM) effectiveness. An et al. (2000) claimed that a polymer-based solution coating would be the most desirable method in terms of stability and adhesiveness of attaching a bacteriocin to a plastic film. It was found that low-density polyethylene (LDPE) films coated with a mixture of polyamide resin in *i*-propanol/*n*-propanol and a bacteriocin solution provided anti-microbial activity against *Micrococcus flavus.*

The potential of incorporating nisin directly into LDPE film for controlling food spoilage and enhancing product safety was highlighted by Siragusa et al. (1999). Devlieghere et al. (2000b) were the first investigators to use hexamethylene-tetramine (HMT) as an anti-microbial packaging agent. Chung et al. (1998) found that LDPE films (48 to 55 µm thick) impregnated with either 1.0 % w/w *Rheum palmatum* and *Coptis chinensis* extracts or silver-substituted inorganic zirconium retarded the growth of total aerobic bacteria, lactic acid bacteria and yeast on fresh strawberries. Preliminary studies by Suppakul and others (2002) with linear low-density polyethylene LLDPE films (45 to 50 µm thick) containing 0.05% w/w linalool or methyl chavicol showed a positive activity in controlling the growth of *E. coli.*

The recent increase in environmental awareness has contributed toward the development of edible packaging materials. Viable edible films and coatings have been successfully produced from whey proteins; their ability to serve other functions, viz. carrier of antimicrobials, antioxidants, or other nutraceuticals, without significantly compromising the desirable primary barrier and mechanical properties as packaging films, will add value for eventual commercial applications in food industries (Ramos et al., 2012). Edible films and various antimicrobial compounds incorporated in edible food packages have also been investigated (Rodrigues and Han 2000; Coma et al., 2001; Appendini and Hotchkiss, 2002). Rodrigues and Han (2000) investigated the edible anti-microbial materials produced by incorporating lysozyme, nisin and ethylenediamine tetracetic acid (EDTA) in whey protein isolates (WPI) films. Both lysozyme and nisin-containing films are effective in inhibiting *Brochothri thermosphacta* but fail to suppress *Listeria monocytogenes*. The incorporation of EDTA in WPI films improved the inhibitory effect on *L. monocytogenes* but had a marginal effect only on *E. coli* O157:H7.

Coma et al. (2001) studied the moisture barrier and the anti-microbial properties of hydroxypropyl methyl cellulose (HPMC)-fatty acid films (30-50 µm thick) containing Nisin (105 IU/mL) as the anti-microbial agent and its efficacy against *Listeria innocua* and *Staphilococcus aureus* growth in food products. Stearic acid was chosen as the fatty acid because of its ability to reduce the rate of water vapour transmission. However, it impaired the effectiveness of the film against both strains. This may be explained by electrostatic interaction between the cationic nisin and the anionic stearic acid.

Foods with different biological and chemical characteristics are stored under different environmental conditions, which, in turn may cause different patterns of microflora growth. The

interactions between the package coatings and anti-microbial agents (AM) are important. For example, aerobic microorganisms can exploit headspace oxygen for their growth. The mechanism and kinetics of growth inhibition are generally studied in order to permit mathematical modelling of microbial growth. The pH of a product was shown to affect the growth rate of target microorganisms and changes the degree of ionization of the most active chemicals, as well as the activity of the antimicrobial agents (Han, 2000). Tobias et al. (2000) reported that LDPE film containing benzoic anhydride was more effective in inhibiting molds at low pH values. Guillard et al. (2009) found that the diffusion of sorbic acid decreased with an increase in pH. The food water activity may also alter the microflora, AM activity, and chemical stability of active ingredients that are applied by impregnation (Kasapis et al., 2009). Rojas-Grau et al. (2008) showed that the diffusion of potassium sorbate through polysaccharide films increases with water activity and this has a negative impact on the amount available for protection.

Anti-microbial packaging is a rapidly emerging technology. The need to package foods in a versatile manner for transportation and storage, along with the increasing consumer demand for fresh, convenient, and safe food products presages a bright future for anti-microbial packaging (Floros et al., 1997). However, more information is required on the chemical, microbiological and physiological effects of these systems on the packaged food especially on the issues of nutritional quality and human safety (Floros et al., 1997). Current research on anti-microbial packaging has focused primarily on the development of various methods and model systems, whereas little attention has been paid to its preservation efficacy in actual foods (Han, 2000; Cha and Chinnan, 2004). Research is essential to identify the types of food that can benefit most from AM packaging materials. It is likely that future research into a combination of naturally-derived AM agents, biopreservatives and biodegradable packaging materials will highlight a range of the merits of AM packaging in terms of food safety, shelf-life and environmental friendliness (Nicholson, 1998; Rodrigues and Han, 2000; Coma et al., 2001).

The storage temperature may affect the activity of AM packages. Several researchers found that the protective action of AM films deteriorated at higher temperatures, due to high diffusion rates in the polymer (Wong et al., 1996). The diffusion rate of the AM agent and its concentration in the film must be sufficient to remain effective throughout the shelf life of the product (Cooksey, 2000).

Polymer nanotechnology in packaging

The worldwide sales of nanotechnology products to the food packaging sector rose from US$ 150 million in 2002 to US$ 860 million in 2004 and has risen steadily (Verbeke, 2006; Meetoo, 2011). There are new innovations to encourage active packaging which involves the combination of food-packaging materials with antimicrobial substances such as the incorporation of antibacterial nanoparticles into polymer films to control microbial surface contamination of foods. It was observed for both migrating and non-migrating antimicrobial materials, an intensive contact between the food product and packaging material is required and therefore potential food applications include vacuum or skin-packaged products, e.g. vacuum-packaged meat, fish, poultry or cheese.

Nanocomposites are known to exhibit increased barrier properties, increased mechanical strength, and improved heat resistance compared to their neat polymers and conventional composites (Sorrentino et al., 2007). Nanoclays, kaolinite, carbon nanotubes and graphene nanosheets that are used as fillers were shown to have potentials that will improve the ability of plastic packaging against migration of gases and flavour compounds, as well as boosting shelf life (Arora and Padua, 2010).

Cellulose, polylactic acid (PLA) have received attention as sustainable, biocompatible, biodegradable materials with good mechanical and optical properties. Lactic acid, the monomer of PLA, may easily be produced by fermentation of carbohydrate feedstock such as corn. Thus, PLA offers more disposal options and its manufacture is less environmentally burdensome than traditional petroleum-based plastics (Arora and Padua, 2010). There are also possibilities to combine antimicrobial compounds with different types of carriers (plastic and rubber articles, paper-based materials, textile fibrils and food-packaging materials). Antibodies may also be attached to fluorescent nanoparticles to detect chemicals or foodborne pathogens. A successful polymer nanotechnology in food packaging will have to take into consideration the complete life cycle of the packaging material (Silvestre et al., 2011). The life cycle assessment consider the overall impact on the environment from all the stages of raw materials sourcing to the production process, transportation and delivery until it reaches end users and finally being disposed (Chaffee and Yoros, 2007). The

sustainability goal inherent within the cradle-to-cradle concept (imposing zero impact on future generations) builds on life cycle analysis to address the material and energy recovery (McDonough and Braungart, 2002). Furthermore, new packaging materials are being developed to facilitate the goal of true sustainability

Multidisciplinary approach to solve future problems

A symposium devoted to the "Plastic Packaging of Foods - Problems and Solutions" identified plastics as the consumer preference of tomorrow and suggested that consumers need to be provided with packages that are economic, convenient and environmentally sound. The major demands by consumers which are still relevant today were identified as - convenience, quality, safety and recyclability (Fox, 1989).

Convenience: Consumers demand products and packaging that make life easier and allow them to enjoy more available leisure time. This convenience applies to closure systems, consumers look for easy open ends, dispensing closures and re-sealable packaging.

Quality: Consumers are usually willing to pay for high quality products they can rely on. Aseptic packaging, irradiation processing and controlled atmosphere packaging are examples of innovations that enhance product shelf life and quality.

Safety: With more dual career families, children are playing an ever larger role in the home, and consumers are looking for packaging that is shatter resistant and easy for children to use. A substantial majority of consumers are willing to pay extra for tamper evident packaging.

Recyclabilty: Consumers want packaging materials that are environmentally friendly.

In the past twenty three years after the 1989 symposium, the production and the use of plastics in the world have been enormously increased, worsening the problem of the waste disposal. The growing interest in environmental impact of discarded plastics has directed research on the development of plastics that degrade more rapidly in the environment, leading to a complete mineralization or bioassimilation of the plastics (Mergaert and Swings, 1996; Tokiwa et al., 2009, Thompson et al., 2009). Biopolymers should be used in those applications where biodegradability and/or the derivation of natural resources gives added value, particularly, where valuable petroleum-based plastics are used for applications with a short life time.

Currently, WikiCells have just been developed at Harvard University; they are novel edible forms for eating and drinking transportable foods and drinks without plastic and would help to reduce waste. They use special membrane technology that permits the fabrication of thin delicious membranes with significant water diffusional resistance and adjoined shells that allow for stability of the WikiCells over long periods of time (WikiCells report, 2012).

There are health concerns regarding residual monomer and components in plastics and paper, including stabilizers, plasticizers, and condensation components such as bisphenol A (BPA). Some of these concerns are based on studies using very high intake levels; others have no scientific basis. The active form of BPA binds to the steroid receptors and can affect estrogen, thyroid and testoterone functions (Science Daily report, 2011). In order to ensure public safety, national and international regulatory bodies such as the Food and Drug Administration (FDA), European Food Safety Authority (EFSA) carefully reviews and regulates substances used to make plastics and other packaging materials. Any substance that can reasonably be expected to migrate into food is classified as an indirect food additive subject to regulations. The Swedish government recently introduced a ban on bisphenol A in food packaging intended for children under the age of three from the beginning of 2013 (Food Production Report, 2012).

There was also a recent study about the effects of chemicals such as perfluorinated compounds (PFC) which are widely used in food packaging. They are found in teflon cookware, microwave popcorn bags and stain-resistant carpets. These chemicals can weaken the ability of vaccination jabs to protect young children. Grandjean et al. (2012) reported that children exposed to perfluorinated compounds (PFCs) in the womb or in the first years of life had lower immunity to tetanus and diphtheria.

The choice of a particular plastic or a flexible package will be linked to developments in engineering and consumer studies. There will continuously be new packaging materials that will reflect developments in the technology of food processing, life style changes, political decision making, and environmental issues. These challenges will be best tackled by multi-disciplinary approach that addresses these issues in the nearest future.

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PLANT SCIENCE

Effect of potassium uptake on the composition of essential oil content in *Calendula officinalis* L. flowers

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Abstract

Potassium (K) is an important macronutrient and the most abundant cation in higher plants. K has been the target of some researchers mainly because it is essential for enzyme activation such as enzyme of essential oil synthesis. The highest accumulation of essential oil (0.29% and 0.095 g plant^{-1}) was recorded at the treatment of 173.2 kg ha^{-1} compared with control treatment (0.13% and 0.015 g plant^{-1}). 28 constituents were identified in the essential oil. The main constituents (α-cadinol, Δ-and γ -cadinene) increased with K level. The highest amount of the main constituents [α-cadinol (33.11%), Δ-cadinene (18.41%), and γ -cadinene (9.99%)] was produced from the F treatment (346.4 kg ha^{-1}). Alcohols were the major constituents of the heavy oxygenated compounds (HOC) of *Calendula* essential oil in which α-Cadinol represented the highest concentration.

Key words: *Calendula officinalis* L., Essential oil, Potassium (K), α-cadinol, Δ-and γ –cadinene

Introduction

Calendula officinalis L. (English marigold, pot marigold) is an annual plant belonging to the *Asteraceae* (*Compositae*) family with bright yellow or orange daisy-like flowers which are used for medicinal or culinary purposes (Beerentrup and Robbelen, 1987; Cromack and Smith, 1988). *C. officinalis* can be broadly applied as an antiseptic, anti-inflammatory and cicatrizing (Correa Júnior, 1994) as well as a light antibacterial and antiviral agent (Chiej, 1988). Many *Calendula* species have a characteristic scent or taste caused by mono- and sesquiterpenes within the essential oil, which in many cases are the reason for their application in folk medicine (Msayuki et al., 2001). Recently, many attempts have been made to better characterize their therapeutic properties and to enhance the production of these useful compounds within their essential oils. Selected *Calendula* chemotypes growing in soil or *in vitro*, for example, flowers of the cadinol chemotype, are very important in European and western Asian folk medicines and are used to treat inflammatory conditions (Msayuki et al., 2001).

Plant nutrition is one of the most important factors that increase essential oil production. Potassium (K) is an important macronutrient and the most abundant cation in higher plants. K has been the target of some researchers mainly because it is essential for enzyme activation such as enzyme of essential oil synthesis (Machner, 2001; Silva, 2004). High K level decreased the essential oil content of bracts and leaves of *Origanum dictamnus* L. plants (Economakis, 1993).

Hornok (1980) indicated that K fertilization was effective on the essential oil content of dill (*Anethum graveolens* L.) while, Hussien (1995) reported that K fertilization was more affected on the dill essential oil constituents. Also, K fertilization increased the essential oil content of some medicinal *Apiaceae* plants (Khalid, 1996). K uptake in *Tagetes minuta* L. plants increases essential oil content (Somida, 2002). Kahlid et al (2007) indicated that fertilization with potassium affected essential oil and its constituents in rue (*Ruta graveolens* L.). The highest content of peppermint (*Mentha piperita*) essential oil production was obtained with fertilization with K at 218 mg L^{-1} (Mollafilabi et al., 2010). Furthermore, addition of K at 123 kg ha^{-1} year^{-1} enhanced the total essential oil yield of palmarosa (*Cymbopogon*

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martinii [roxb.] wats. var. motia burk) plant by 23.7% in comparison to no K application (Munnu, 2008). Similarly, K fertilization increased essential oil and anethole contents of anise (*pimpinella anisum* L.) plant (Al-Awak, 2010). When treated with different concentrations of K, caraway (*Carum carvi* L.) experienced an increased in essential oil content, linalool, and limonene but no considerable differences was observed in carvone (Ezz El-Din et al., 2010). In this study, we investigate the possible effect of K on the essential oil content of *C. officinalis* flower heads.

Materials and Methods

Experiments were carried out at the National Research Centre (NRC), Giza, Egypt under natural coditions, for two seasons (2007/2008 and 2008/2009). *C. officinalis* seeds were obtained from the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Giza, Egypt. In the first week of November during both seasons, seeds were sown in plastic pots (30 cm diameter and 50 cm height) as 10 seeds per pot. The viability of the seeds was approximately 92%. In the third week of December during both seasons, the pots were transferred to natural conditions within the greenhouse of the NRC were each pot was filled with 10 kg of air-dried clay loam soil (meteorological data at Giza, Egypt during the two growing seasons are presented in Table 1). Physical and chemical properties of the soil used in this study were determined according to Jackson (1973) and Cottenie et al. (1982) and are presented in Table 2. Eight weeks after sowing, the seedlings were thinned to three plants per pot. All agriculture practices operations other than experimental treatments were done according to the recommendations of Ministry of Agriculture, Egypt. Pots were divided into 6 main groups adjusted to K_2SO_4 (48%, K_2O) as a source of potassium with varying amounts of K as [A] 0 (control), [B] 115.5, [C] 173.2, [D] 231.00, [E] 288.7 and [F] 346.4 kg ha^{-1} (as K_2O form) respectively.

Harvesting

Fresh flower heads were collected from each treatment at flowering stages during both seasons and air dried. The flower yield (g $plant^{-1}$ of fresh and dry weights of flower heads) was recorded.

Essential oil isolation

Fresh flower heads were collected from each treatment and from the flowering stage in both seasons, air dried and weighted to extract the essential oil. Dry flower heads (500 g) from each of these treatments was hydro-distilled for 3 h using a Clevenger-type apparatus (Clevenger, 1928). The essential oil content was calculated as a percentage. Also g essential oil $plant^{-1}$ was calculated according to the dry weights of flower heads per plant.

Table 2. Physical and chemical properties of the soil.

Sand (%)	26
Silt (%)	36
Clay (%)	38
Texture class	Clay loam
CaCo3 (%)	4.5
O.M.	1.3
pH	1.7
Soluble cations and anions (mequiv./l)	
EC	0.57
Na^{+}	2.23
Mg^{++}	0.88
Ca^{++}	1.11
K^{+}	1.48
$HCO3^{-1}$	1.12
$Co^{+}3$	0.73
Cl^{-1}	2.10
$SO4^{-2}$	1.62

GC/MS: The essential oil was analyzed on a VG analytical 70- 250S sector field mass spectrometer, 70 eV, using a SPsil5, 25 m x 0.30 mm, 0.25 µm coating thickness, fused silica capillary column, injector 222°C, detector 240°C, linear temperature 80°–270°C at 10°C/min. One microliter of diluted essential oil (1/100, v/v, in *n*-pentane) was injected manually in the GC at 250 °C, with the splitless mode flame ionization detection (FID) using the HP Chemstation software on a HP 5980 GC with the same type column as used for GC/MS and same coditions program.

Qualitative and quantitative analyses essential oil

Identifications of essential oilcomponents were made by library searches (Adams, 2007) combining MS and retention data of authentic compounds and comparison of their GC retention indices (RI) with those of the literature (Adams, 2007). The retention indices were determined in relation to a homologous series of *n*-alkanes (C8–C22) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 98 and Wiley 5 Libraries or with mass spectra from literature. Component relative concentrations were calculated based on GC peak areas without using correction factors.

Table 1. Meteorological data at Giza, Egypt during the two growing seasons.

Months	1st season (2007/2008)					2nd season (2008/2009)				
	T (°C)		Rs (MJm $^{-2}$ d $^{-1}$)	RH (%)	ETp (mmd $^{-1}$)	T (°C)		Rs (MJm $^{-2}$ d $^{-1}$)	RH (%)	ETp (mmd $^{-1}$)
	Max.	Min.				Max.	Min.			
November	22.6	10.5	13.0	48.2	23.1	22.8	12.0	12.2	38.9	24.2
December	19.4	11.6	14.4	48.9	27.4	21.3	11.5	13.5	40.2	26.8
January	18.0	10.9	24.9	48.3	28.3	19.6	10.2	22.6	49.3	32.5
February	23.8	12.6	25.9	55.6	29.6	22.1	16.2	26.9	50.2	33.5
March	27.5	14.3	26.7	70.6	30.4	28.7	17.6	27.2	52.4	34.0
April	28.9	14.4	27.5	80.4	36.5	29.1	18.1	30.5	56.2	37.5
May	31.2	16.3	31.6	88.9	42.5	30.4	20.1	32.5	74.2	44.2
June	35.4	20.5	35.8	90.3	49.6	33.8	21.3	33.8	84.2	51.1

Monthly average. T: temperature; Rs: solar radiation; RH: relative humidity; ETp: potential evapotranspiration.

Statistical analysis

In this experiment, 1 factor was considered: potassium amount (control, 115.5, 173.2, 231.00, 288.7 and 346.4 kg ha^{-1}). For each treatment there were 4 replicates, each of which had 10 pots; in each pot 3 individual plants were planted. The experimental design followed a complete random block design according to Snedecor Cochran (1990). The flower samples were collected from each replicate (30 plants). The averages of data were statistically analyzed using analysis of variance one - way (ANOVA-1). Significant values determined according P values ($P<0.05$ = significant, $P<0.01$ = more significant and $P<0.001$ = highly significant). The applications of that technique were according to the STAT-ITCF program (Foucart, 1982).

Results

Effect of fertilizing potassium amount on the essential oil content

As shown in Table 3, essential oil yield increased with the potassium level compared with control treatment. The highest accumulation of essential oil (0.29% and 0.095 g $plant^{-1}$) was recorded at potassium treatment of 173.2 kg ha^{-1}, compared with that of the control treatment (0.13% and 0.015 g $plant^{-1}$). The yield of essential oil yield was low at potassium treatments 115.5, 231.00, 288.7 and 346.4 kg ha^{-1} compared with that of treatment 173.2 kg ha^{-1}. The changes in essential oil yield were highly significant ($P<0.001$) for K treatments (Table 3).

Table 3. Yield (% and g $plant^{-1}$) of *Calendula officinalis* L. flower heads essential oil after fertilization with different concentrations of potassium.

K_2O_5 (kg ha^{-1}) treatments	Essential oil (%)	Essential oil (g $plant^{-1}$)
Control	0.13	0.015
115.5	0.18	0.035
173.2	0.29	0.095
231.0	0.24	0.054
288.7	0.25	0.051
346.4	0.26	0.052
F ratio*	22.31***	840.84***

* $P \leq 0.05$ (significant) according to F-values of the 2-way analysis of variance (ANOVA-2).

** $P < 0.01$ (more significant) according to F-values of the 2-way analysis of variance (ANOVA-2).

*** $P < 0.001$ (highly significant) according to F-values of the 2-way analysis of variance (ANOVA-2).

F ratio* = Calculated F

Effect of K on the chemical composition of essential oil

Table 4 shows that 28 constituents, amounting 99.20 - 99.64% of the essential oil were identified. The main constituents of *Calendula* essential oil as detected by GC-MS were α-cadinol, Δ-and γ - cadinene. The highest values of the main components (33.11, 18.41, both at 9.99%) were obtained from potassium treatment of 346.4 kg ha^{-1}. The relative level for other various constituents were changed (increased or decreased) in *C. officinalis* flower heads under different K treatments compared with untreated control treatment.

Table 4 included the compounds obtained from *C. officinalis* essential oil under K treatments grouped into classes (monoterpenes, sesquiterpenes, light oxygenated compounds, heavy oxygenated compounds) and their percentages compared with the control treatment. From the same table, it is noticeable that the concentration of heavy oxygenated compounds was the highest in essential oil with all in plants treated with K compared to the control and other chemical classes (light oxygenated compounds, sesquiterpene and monoterpene). Alcohols were the major constituents of heavy oxygenated compounds in *Calendula* essential oil with α-Cadinol as the highest concentration among these alcohols. This indicates that most of *Calendula* essential oils, the experimental conditions belong to the α-cadinol chemotype. The highest amount of light oxygenated compounds (11.31%) resulted from the control treatment; the highest amount of sesquiterpenes (46.53) resulted from the 173.2 kg ha^{-1} treatment; the highest amount of monoterpene (1.78) resulted from the 231.0 kg ha^{-1} treatment; while the highest amount of heavy oxygenated compounds (46.50) resulted from the 346.4 kg ha^{-1} treatment. The main components of essential oil (α-cadinol, γ-and Δ-cadinene) increased with the potassium level compared with control treatment. ANOVA indicated that the changes in all essential oil constituents were highly significant for K treatments except for aromadendrene (allo) [more significant ($P<0.01$)] and α-cadinol (insignificant). On the other hand, changes in monoterpene and sesquiterpenes were highly significant ($P<0.001$) while it was more significant in light oxygenated compounds but it was insignificant heavy oxygenated compounds for K treatments (Table 4).

Table 4. Effect of potassium on the essential oil constituents extracted from *Calendula officinalis* L. flower heads.

No	Components (%)	KI*	K_2O_5 (kg ha $^{-1}$) treatments						*F -ratio*
			Control	115.5	173.2	231.0	288.7	346.4	
1	α-Pinene	938	1.75	1.32	1.33	1.78	0.63	1.39	77.65***
2	β-Farnesene	1450	0.19	0.22	0.35	1.56	1.43	1.32	1753.95***
3	α-Humulene	1455	0.22	1.12	0.89	1.67	0.88	0.98	2613.60***
4	α-Patchoulene	1457	0.22	1.12	0.78	1.66	1.98	2.11	3314.47***
5	Aromadendrene	1463	1.20	1.23	1.23	1.12	1.13	1.34	8.85**
6	γ-Gurjunene	1474	2.41	2.34	1.34	1.11	1.89	1.63	1135.10***
7	γ-Muurolene	1478	0.43	1.56	0.78	1.14	1.96	1.37	4526.20***
8	α-Muurolene	1500	0.55	0.34	0.67	0.45	0.87	0.55	80.68***
9	γ-Cadinene	1514	9.74	10.34	11.89	9.88	9.96	9.99	3231.40***
10	Δ-Cadinene	1523	17.80	18.33	20.67	17.97	18.38	18.41	1731.88***
11	β-Patchouli alcohol	1539	3.41	3.44	2.16	2.67	1.98	1.67	3729.80***
12	α-Calcorene	1541	1.72	1.13	1.34	1.89	1.32	1.38	413.21***
13	Muurol-5-enen-4-B-oL	1546	1.32	1.34	1.22	1.65	0.57	0.45	643.36***
14	β-Calacorene	1564	3.24	2.34	1.45	1.76	0.98	1.23	1842.32***
15	Nerolidol (*E*)	1565	5.70	3.45	1.67	1.12	1.15	1.11	4011.6***
16	Guaiol	1596	1.14	1.13	1.32	1.67	1.98	1.76	1501.92***
17	β-Acorenol	1635	3.61	2.24	2.67	2.22	2.65	2.13	1521.33***
18	Cadinol (epi-α)	1639	0.86	1.45	1.12	2.24	2.87	2.65	1681.99***
19	Muurolol	1640	0.13	0.17	0.16	1.34	1.87	1.33	4093.32***
20	α-Eudesmol	1653	2.26	1.29	1.34	0.98	1.22	1.24	844.92***
21	ά-Patchouli alcohol	1654	1.62	1.54	1.34	1.96	2.23	2.39	812.02***
22	Bulnesol	1658	1.28	1.39	1.82	1.44	1.78	1.95	408.87***
23	α-Cadinol	1663	32.00	32.37	34.76	32.78	32.56	33.11	0.71
24	Eudesmol	1669	0.96	1.11	1.23	1.76	1.56	1.99	2063.70***
25	Isocedranol	1670	0.75	0.77	0.69	1.55	1.35	1.37	315.00***
26	α-Bisabolol	1684	1.72	1.69	1.11	1.23	1.26	1.38	33.77***
27	Bisabolol	1687	0.27	1.45	1.23	1.13	1.45	1.29	518.68***
28	Pentacosane	2501	2.80	2.98	2.98	1.87	1.42	2.12	682.94***
Total identified			99.30	99.20	99.54	99.60	99.31	99.64	
Monoterpenes			1.75	1.32	1.33	1.78	0.63	1.39	648.46***
Sesquiterpenes			43.93	46.49	46.53	44.75	44.18	44.10	215.77***
Light oxygenated compounds			11.31	8.27	6.78	7.25	8.65	7.65	5.86**
Heavy oxygenated compounds			42.31	43.12	44.90	45.82	45.85	46.50	1.00

* $P \leq 0.05$ (significant) according to F-values of the 2-way analysis of variance (ANOVA-2).

** $P < 0.01$ (more significant) according to F-values of the 2-way analysis of variance (ANOVA-2).

*** $P < 0.001$ (highly significant) according to F-values of the 2-way analysis of variance (ANOVA-2).

KI*= Confirmed by comparison with Kovats indices on DB5 column (Adams, 2001).

Discussion

In this study, potassium fertilization increased the essential oil content. The results were confirmed by previous literature (Hornok, 1980; Hussien, 1995; Khalid, 1996) in which it was reported that K fertilization increased the essential oil content of plants. All potassium treatment concentrations used in this study induced low essential oil content except for the treatment of 173.2 kg ha^{-1}, in agreement with Economakis (1993) who indicted that high K level decreased the essential oil content of bracts and leaves of *Origanum dictamnus* L. plants.

The quality of the essential oil used in this investigation was somewhat similar to that of a French *Calendula* chemotype whose essential oil composition was rich in α-cadinol (Chalchat et al., 1991). We noticed that the changes in the chemical constituents *of C. officinalis* essential oil were confirmed by previous literature (Munnu, 2008; Al-Awak, 2010; Ezz El-Din et al., 2010). Claimed that within the essential oil , the relative level of various constituents increased, decreased, or did not change at all in mint (*Mentha* sp.), palmarosa (*Cymbopogon martinii* [roxb.] wats. var. motia burk) and caraway plants under K treatments, compared with control plants (Munnu, 2008; Mollafilabi et al., 2010). The different essential oil yield and constituents as a result of potassium treatment may be due to their effects of potassium on enzyme activity and

metabolism during essential oil production by the plants (Burbott and Loomis, 1969; Machner, 2001; Silva, 2004). α-Cadinol was reported to be up to 26.38% in this French ecotype (Chalchat et al., 1991). Previously, the essential oil of French *C. officinalis* was obtained in low yield (0.3%) by steam distillation of flowers heads, and 66 components were identified by GC-MS, mainly sesquiterpene alcohols; α-cadinol was the main constituent (Chalchat et al., 1991). The essential oil concentrates of *C. officinalis* flower heads extracted by supercritical CO_2 was found to consist of methylhexanoate, methyllinoleate, methyl 9, 12, 15-octadecatrienoate, methyloctadecanoate, methyl-tetradecanoate, γ-cadinene and cubenol, δ-cadinene, α-cadinol and oplopanonec (Nicoletta et al., 2003). The main constituents of *C. officinalis* essential oil as detected by GC-MS and cultivated under presowing low temperature were α-cadinol, Δ-cadinene, δ-cadenene and nerolidol (Khalid and El-Ghorab, 2006). The main constituents (α-cadinol, Δ-cadinene) of essential oil extracted from whole *C. officinalis* plants and detected by GC-MS increased when organic fertilizers were applied under soil solarization conditions (Khalid et al., 2006). The main constituents of *C. officinalis* essential oil as detected by GC-MS and cultivated under saline conditions were α-cadinol, Δ-cadinene, δ-cadenene (Khalid and Teixeira da Silva, 2010).

Hornok (1980) indicated that K fertilization was effective on the essential oil content of dill (*Anethum graveolens* L.) while, Hussien (1995) reported that K fertilization was more affected on the dill essential oil constituents. Also, K fertilization increased the essential oil content of some medicinal *Apiaceae* plants (Khalid, 1996). K uptake in *Tagetes minuta* L. plants increases essential oil content (Somida, 2002). Kahlid et al (2007) indicated that fertilization with potassium affected essential oil and its constituents in rue (*Ruta graveolens* L.). The highest content of peppermint (*Mentha piperita*) essential oil production was obtained with fertilization with K at 218 mg L^{-1} (Mollafilabi et al., 2010).

Conclusion

Essential oil yield increased with the potassium level compared with control treatment. The highest accumulation of essential oil was recorded at potassium treatment of 173.2 kg ha^{-1}. Alcohols are the major constituents of the heavy oxygenated compounds (HOC) of *Calendula* EO with α-Cadinol as the main component representing the highest concentration among the alcohols. This indicates that *Calendula* essential oil obtained under growth under potassium belongs to the α-cadinol chemotype. The main constituents (α-cadinol, Δ-and γ -cadinene) increased with K level. Heavy oxygenated compounds were the highest in essential oil with all in plants treated with K compared to the control and other chemical classes (light oxygenated compounds, sesquiterpene and monoterpene). Alcohols were the major constituents of heavy oxygenated compounds.

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PLANT SCIENCE

Virulence variation in *Alternaria mali* (Roberts) and evaluation of systemic acquired resistance (SAR) activators for the management of Alternaria leaf blotch of apple

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Abstract

Alternaria leaf blotch, caused by *Alternaria mali*, is an economically important disease of apple (*Malus* x *domestica* Borkh.). Twenty one isolates of *Alternaria mali* (Am-1 to Am-21) were obtained during the isolate collection. The virulence was tested on detached leaves of susceptible Red Delicious cultivar and Am-1 showed highest virulence. Two greenhouse trials were conducted using two year old grafted seedlings of Red Delicious apple cultivar to assess the efficacy of seven SAR activators, 2,6-dichloroisonicotinic acid (INA), benzothiadiazole S-methyl ester (BTH), β-aminobutyric acid (BABA), K_2HPO_4, K_3PO_4, $Ca(OH)_2$ and $CaCO_3$ applied 48 hrs before and after spore inoculation. The SAR activators and a conventional synthetic fungicide (penconazole) were evaluated against most virulent isolate Am-1. Distilled sterilized water was sprayed on control plants. All the SAR activators significantly lowered the disease intensity as compared to control. BABA was most effective with least disease intensity before and after pathogenic inoculation. Penconazole proved superior to all the SAR activators, except BABA. The application of SAR activators before pathogen inoculation showed significantly lower disease intensity (12.71%) in comparison to SAR application after pathogen inoculation (14.77%). This induced resistance exploiting natural defense machinery of plants could be proposed as a non-conventional and eco-friendly approach for plant protection.

Key words: Apple, Alternaria leaf blotch, Virulence variation, Detached leaf technique, SAR Activators

Introduction

The apple (*Malus* x *domestica* Borkh.) is the most ubiquitous of temperate fruits cultivated in Europe and Asia from antiquity. India ranks 7th with an annual production of 2163400 MT of apple fruit (FAO, 2012). In India apple is predominantly grown in Himalayan states like Jammu and Kashmir (J&K), H.P. and Uttaranchal which account for about 90% of the total production of country (Anonymous, 2002). Like other horticultural crops apple is attacked by several pathogens which impair the quality and quantity of the fruit. However, huge losses of the crop are incurred mostly by fungal diseases. The major fungal diseases include scab, Alternaria leaf blotch, powdery mildew, collar rot, root rot, sooty blotch and fly speck etc. Amongst these, Alternaria leaf blotch caused by *Alternaria mali* is prevalent in all the apple growing areas of world and is one of the economically important apple diseases. In J&K, the occurrence of disease (*Alternaria mali*) was reported by Shahzad et al. (2002). The disease previously considered to be of minor significance has now attained the status of major disease (Anonymous, 2000) and is prevalent in almost all apple orchards of Kashmir valley with a potential threat to existing apple plantation. One of the significant aspects of the biology of an organism is the morphological and physiological characters of an individual within a species which are not fixed. This holds true with fungi also, thus variability studies are important to document the changes occurring in the population and individuals. The variability is a well-known phenomenon in genus *Alternaria* and such variability may be seen in virulence also. Shahzad (2003) reported prevalence of Alternaria leaf blotch of apple in all the districts of Kashmir with varied degree of incidence and

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intensity. Variation in susceptibility among different apple cultivars was also recorded. This variation may be attributed to many factors including variability in pathogen. Understanding population structure of pathogen is of paramount importance for devising a successful disease management programme. Thus variation in the virulence was studied and the most virulent isolate was evaluated against the SAR activators.

Among the various disease management strategies, chemical application still dominates our approach involving frequent fungi-toxicant interventions throughout the world (Koller et al., 2004). Kashmir valley with temperate environmental conditions is a favourable place for the development of fungal diseases. To combat these diseases a need-based spray schedule comprising 6-8 sprays of fungi-toxicants is in vogue (Anonymous, 2002). This poses serious environmental concerns and selection pressure on pathogen population. The development of fungicide resistance in pathogenic populations is one of the most serious constraints because substantial changes in the populations of several major plant pathogens in their sensitivity to fungicides have been observed. This frequently leads to significant crop damage and forcing either discontinuation or modification in the use of important chemicals. The frequent and successive use of fungi-toxicants is likely to develop resistance among the pathogen (Koller et al., 2004; Thind, 2008) and as such threaten the success of spray programme due to the occurrence of resistance in the pathogen (Koller and Wilcox, 1999; Thind, 2008). Some isolates of *Alternaria mali* have developed tolerance to fungicides like iprodione, mancozeb and captan (Lee and Kim, 1986; Osanai et al., 1987; Asari and Takahashi, 1988). The use of resistant cultivars is an alternative to minimize the disease. However, in apple it is difficult to develop resistant cultivars because of long juvenile phase and self-incompatibility. Hence, use of SAR activators in the disease management seems environmentally safe as compared to current pesticides (Vallad and Goodman, 2004). Unlike traditional pesticides, SAR activators do not exhibit any direct antimicrobial activity but prime the plants for resistance. Thus provide a safe way to control disease without asserting direct selection pressure on pathogen populations. SAR is based on multiple natural defense mechanisms, and this makes it less likely that a pathogen can readily develop resistance to this control measure. Over the last 30 years a number of compounds (SAR activators) have been shown to be instrumental in enhancing disease resistance or at least decreasing the disease symptoms in plants. The use of SAR activators for disease control is safe application because SAR activators neither have any toxic effect on pathogens, plants and animals, nor show any inhibitory effect on plant growth, development and yield. Increased pathogen insensitivity to synthetic fungicides coupled with public demands to reduce pesticide use, stimulated by greater awareness of environmental and health issues has placed more emphasis on alternative pathogen control strategies.

Since the crop and disease are of paramount importance to J & K state (India) and no such studies of virulence variation in *Alternaria mali* isolates and management through eco-friendly approaches (SAR activators) have been conducted in the State, therefore the present study was undertaken with the objectives:

To study virulence variation and ascertain most virulent isolate in *A. mali* and to evaluate SAR activators against the most virulent isolate.

Materials and Methods

Collection of isolates

Apple leaves from susceptible cultivar (Red Delicious) exhibiting typical symptoms of Alternaria leaf blotch were collected from twenty one apple orchards across seven districts of Kashmir valley, *viz*., Bandipora, Baramulla, Budgam, Ganderbal, Kulgam, Pulwama and Shopian. For isolation of fungal pathogen, the diseased leaf area along with some healthy portion was cut into small bits with a sharp sterilized blade. These bits were surface disinfected in 0.1 per cent mercuric chloride ($HgCl_2$) for about 30 seconds followed by three washing in sterilized distilled water to remove the last traces of $HgCl_2$. After blotting dry with sterilized filter papers, these bits were transferred to sterilized potato dextrose agar medium (PDA) in sterilized petriplates. Three such bits were placed in each petriplates and incubated for seven days at 24±1°C. The isolates were purified through single spore isolation technique (Johnston and Booth, 1983) by transferring germinating conidia to petriplates containing sterilized PDA medium. On the basis of cultural and morphological characters, establishment of pathogenicity and comparison with authentic description (Roberts, 1924; Shahzad, 2003), the fungus was identified as *Alternaria mali*. The pure cultures were maintained on PDA slants and stored at 4°C in a refrigerator. The isolates thus obtained were designated as Am-1 to Am-21.

Determination of the virulence of Alternaria mali isolates

The virulence of *Alternaria mali* isolates (Am-1 to Am-21) was tested on detached leaves of susceptible Red Delicious cultivar through "Detached leaf technique" as follows:

Preparation of inoculum

The isolates of *A. mali*, were multiplied on PDA medium for 10 days at 24±1°C in BOD (Biological oxygen demand) incubator. After 10 days of incubation, spore suspension was prepared by flooding culture plate with sterilized distilled water. Culture plates were scraped with a sterilized razor blade, strained through a double layer of sterile cheesecloth into a 150 ml flask. Spore concentration was adjusted with haemocytometer to 4 x 10^5 conidia per millilitre. The prepared conidial suspensions were preserved at -80°C until use.

Inoculation method

In order to work with a highly virulent isolate, twenty one isolates of *Alternaria mali* were tested. Healthy leaves were collected, washed thoroughly under running water, followed by three washings in sterilized water. After mild rinsing of leaf surface with sterilized water, the leaves were dried with a laboratory towel and placed in petriplates. Each petriplate was lined with a wet blotter paper on the bottom for maintaining high humidity. A set of three leaves, was placed in each petriplate. Each set was replicated thrice. The leaves were inoculated (outside the petriplates) with a conidial suspension (4×10^5 spores/ml) with an atomizer to the whole surface. Before inoculation celite (0.5%) was applied to the leaves with the help of a brush. Leaves in the petriplates were incubated at 24±1^{0}C in BOD incubator with 12 hour dark and 12 hour light adjustment and monitored for symptom development. Ten days after inoculation, number of lesions and size of lesions were recorded. The size of lesion was recorded by taking two perpendicular measurements and their average was calculated, while as total lesion area was calculated by multiplying number of lesions with size of lesions. Isolate which showed highest virulence (highest total lesion area) was selected for further studies. The experiment was laid in CRD (completely randomized design) with three replications.

Evaluation of SAR chemicals

Plant material

Two year old grafted seedlings of Red Delicious apple cultivar obtained from Division of Pomology, SKUAST-Kashmir were used as host plants for the studies. This cultivar is highly susceptible to Alternaria leaf blotch caused by *Alternaria mali*.

Greenhouse conditions

Seedlings were grown in pots of 30 cm x 30 cm size, filled with clay loam soil in the greenhouse. The greenhouse was maintained on temperatures of 25 ± 5°C, humidity of 68-80%, and 12 hourly light. The plants were used 8 weeks after planting (young shoots were 10-15 cm long with 6-10 leaves per shoot). This environment was maintained during the entire period of the experiment.

Application of SAR chemicals

Seven SAR inducing compounds *viz*: INA, BTH, BABA, K_2HPO_4, K_3PO_4, $Ca(OH)_2$ and $CaCO_3$ were evaluated against the most virulent isolate (Am-1) of the pathogen on potted plants cv. Red Delicious under greenhouse conditions. Test plants were divided in two sets each for chemical sprays 48 hrs before spore inoculation and 48 hrs after spore inoculation. Inoculum was provided to the test plants by spraying with spore suspension of ten days old culture. Spore suspension of 4 x 10^5 conidia / ml was prepared adopting the method as discussed earlier in 'preparation of inoculum'. Tween 20 (0.1%) was added to the inoculum. The plants were inoculated by spraying the spore suspension on both leaf surfaces to the drip point with an atomizer. Before inoculation celite (0.5%) was applied to the leaves of the plants with the help of a brush. SAR chemicals were used at three different concentrations (diluted with distilled sterilized water) as inducing agent by spraying on leaves 48 hours before and after inoculation. INA and BTH were tested at 50, 100 and 200 ppm concentration, BABA at 500, 1000 and 2000 ppm, K_2HPO_4, K_3PO_4, $Ca(OH)_2$, $CaCO_3$ at 25, 50 and 100 mM. Penconazole was tested at 300, 400 and 500 ppm. Distilled sterilized water was sprayed on control plants. Three replications were maintained for each treatment. All the chemicals were sprayed to the runoff with an atomizer. Symptom development was evaluated 15 days after spore inoculation according to a rating system from 0-5 (Filajdic and Sutton, 1991) with slight modification (Table 1). The experiment was laid in Factorial CRD with three replications. The experiment was repeated in the 2nd year.

Per cent disease intensity (PDI) was calculated using Mc Kinney's (1923) formula:

$$\text{Per cent disease intensity} = \frac{\text{Sum of all the numerical ratings}}{\text{Number of leaves examined x maximum disease rating}} \times 100$$

Per cent disease control was calculated using formula as adopted by Burpee and Latin (2008):

Per cent disease control= [(C – T)/C] x 100, where C and T = disease severity in control and treated plots, respectively.

Table 1. Scale (0-5) for disease rating.

Numerical value	Criteria
0	No symptoms
1	0 - 3% leaf area covered with lesions
2	4 - 6% leaf area covered with lesions
3	7 - 12% leaf area covered with lesions
4	13 - 25% leaf area covered with lesions
5	26 - 50% leaf area covered with lesions or chlorotic leaf with petiole infection

Results and Discussion

Virulence variation

In the present study all the isolates were successful in producing the disease lesions with variation in number and size of lesions (Table 2). The number of lesions produced by the isolates varied from 6.3 to 14.3 with the least produced by Am-2 and maximum by Am-16. The minimum lesion size of 2.9 mm was recorded in Am-17 while the maximum of 10.2 mm was observed in Am-1. Our findings are in agreement with Thrall et al. (2005) who found significant differences in the lesion size produced by the *Alternaria brassicicola* isolates on *Cakile maritima*. Kumar (2004) also reported variation in lesion size and lesion number produced by the isolates of *Alternaria triticina*. Among the two isolates of *Alternaria carthami* one produced more severe leaf spots than other when inoculated on safflower cultivars (Rai and Kumari, 2009). However the observations are contradictory to the findings of Quayyum et al. (2005) who did not find any significant variation in the lesions produced by the isolates of *Alternaria panax* on detached leaflets of ginseng. The isolate Am-1 was highly virulent as compared to other isolates with the highest total lesion area of 122.4 mm. Filajdic and Sutton (1992) found all the eight isolates of *A. mali* pathogenic on delicious seedlings with varied virulence. Varma et al. (2006) reported variability in virulence among isolates of *Alternaria solani*. In contrast Van der Waals et al. (2004) found a moderate degree of variation in virulence among different isolates of *Alternaria solani*. It can be argued that variation in the isolates may be inherent since isolates were collected from different sites. Physiological characters are influenced by environmental conditions which may be responsible for such variability. Moreover isolates in these sites may have adapted for many years which may be responsible for this variation. The virulence variation of *A. mali* in Kashmir valley may be because of the mixed cultivars in our orchard eco-system, diversity in sites and selection pressure due to indiscriminate use of fungicides. One more possible explanation for the variation found among isolates of *Alternaria mali* could be natural chance mutations, combined with the fact that the fungus can produce abundant number of spores in a relatively short period of time. Brierley (1920) suggested that variation in fungi imperfecti may be due to mutation, or by splitting of an originally impure genetic constitution or of gametic or somatic segregation from heterozygotes. Variants, considered as due to mutation, were found in single spore cultures of *Alternaria mali* (Roberts, 1924). Thus Am-1 being most virulent isolate was used for the following studies.

Efficacy of SAR activators for the management of disease

All the SAR activators along with fungicide (Penconazole) at three different concentrations before and after pathogen inoculation significantly lowered disease intensity as compared to control (Table 3). None of the treated or control plants died as a result of *Alternaria* attack during the course of the two year study. Likewise none of the SAR agents and fungicide evaluated was phytotoxic to the test seedlings. Pooled data shows that disease intensity ranged from 1.59-18.22 per cent in SAR activators treatments (before inoculation) in comparison to 35.53 per cent in control. Similarly it ranged from 3.94-23.99 per cent after inoculation in comparison to 35.74 per cent in control. Chemically-induced SAR has been found effective against various pathogens (Schneider et al., 1996; Kuc, 2001). The least disease intensity of 3.94 per cent (after inoculation) was recorded in BABA which was at par with penconazole having a disease intensity of 3.76 per cent. Several studies indicated that the SAR compounds are useful in the management of fungal pathogens (Christiansen et al., 1999; Kessmann et al., 1994) with the level of pathogen suppression comparable with synthetic fungicides. Consequently, induced resistance could provide systemic protection against pathogen attack

to substitute for, or supplement control by conventional synthetic fungicides. However penconazole (synthetic fungicides) recorded significantly lower disease intensity than SAR chemicals other than BABA. These findings are in agreement with Agostini et al. (2003) and Percival and Haynes (2008) who reported SAR activators to be generally less effective than standard synthetic fungicides in the control of foliar pathogens. Percival et al. (2009) reported penconazole superior over Messenger (a.i. Harpin protein), *Phoenix* (a.i. Potassium phosphite) and Rigel (a.i. Salicylic acid derivative) in controlling apple and pear scab. The other SAR chemicals in decreasing order of their efficacy were K_3PO_4, BTH, INA, $Ca(OH)_2$, K_2HPO_4 and $CaCO_3$ (Figure 1a). K_3PO_4 and K_2HPO_4 showed 79.85 and 57.17 per cent control in 2009. Gottstein and Kuc (1989) suggests phosphate application for disease control because of the effectiveness of systemic resistance induced by phosphates, their low cost, low animal toxicity, nutrient value and comparative safety for the environment. The BTH which showed 68.82 per cent control has been tested against several other pathogens (Ruess et al., 1995; Oostendrop et al., 1996). In the present findings INA showed 64.97 per cent control which is in agreement with the findings of Kessmann et al. (1994) who reported that the foliar spray of INA reduced fire blight (*Erwinia carotovora*) by 45 per cent compared to non-inoculated control. Calcium based chemicals used in our study were effective in combating the disease. Our results are in agreement with the findings of Yoon et al. (1989) who reported effectiveness of application of calcium compounds against *Alternaria mali* and also found higher calcium content in apple leaves responsible for imparting resistance to *Alternaria mali* than in the leaves of susceptible ones.

Table 2. Virulence variability in *Alternaria mali* isolates.

Isolate	No. of lesions*	Size of lesions (mm)*	Total lesion area(mm)
Am-1	12.0	10.2	122.4
Am-2	6.3	9.1	57.3
Am-3	10.0	4.6	46.0
Am-4	10.0	8.5	85.0
Am-5	8.3	6.1	50.6
Am-6	11.0	3.4	37.4
Am-7	9.6	5.6	53.7
Am-8	11.6	6.2	71.9
Am-9	11.6	5.8	67.2
Am-10	6.6	6.4	42.2
Am-11	12.3	8.3	102.0
Am-12	9.0	4.5	40.5
Am-13	13.0	5.7	74.1
Am-14	12.0	6.2	74.4
Am-15	12.3	3.1	38.1
Am-16	14.3	4.3	61.4
Am-17	6.0	2.9	17.4
Am-18	8.6	5.6	48.1
Am-19	8.3	5.1	42.3
Am-20	11.3	3.3	37.2
Am-21	12.0	4.6	55.2
$CD_{(P = 0.05)}$	2.41	0.74	

*After 10 days of inoculation and mean of three replications

Table 3. Efficacy of SAR activators against *Alternaria* leaf blotch of apple cv. Red Delicious (mean±SD).

Treatment	Conc. (ppm)/ *: mM	Disease intensity (%)					
		2009		2010		Pooled	
		BI	AI	BI	AI	BI	AI
INA	50	14.42±1.60	16.56±0.70	13.95±0.67	15.75±0.63	14.18±0.96	16.15±0.58
(2,6-dichloroisonicotinic acid)	100	11.50±1.16	12.30±1.28	12.05±0.09	12.85±0.29	11.77±0.56	12.57±0.74
	200	9.28±0.72	10.22±0.37	9.30±0.27	9.70±0.35	9.29±0.48	9.96±0.33
BTH	50	11.40±0.95	14.75±1.18	10.93±0.32	13.25±0.52	11.16±0.32	14.00±0.40
(benzothiadiazole-S-methyl ester)	100	9.77±0.43	12.83±0.41	9.20±0.30	10.93±0.43	9.48±0.10	11.88±0.02
	200	7.99±0.42	9.42±0.70	7.50±0.24	10.00±0.74	7.74±0.33	9.71±0.49
BABA	500	9.00±0.42	9.93±0.70	8.55±0.44	9.10±0.11	8.77±0.29	9.51±0.34
(β-aminobutyric acid)	1000	4.65±0.58	6.23±0.54	5.25±0.29	7.05±0.36	4.95±0.28	6.64±0.09
	2000	1.47±1.05	4.03±0.14	1.72±0.41	3.86±0.30	1.59±0.36	3.94±0.21
*K_2HPO_4	25	16.39±1.18	19.37±0.96	17.23±0.28	18.35±0.23	16.81±0.72	18.86±0.38
	50	15.04±1.66	16.24±0.94	14.75±0.31	16.00±0.10	14.89±0.76	16.12±0.44
	100	10.82±1.51	12.97±0.39	10.15±0.34	12.34±0.32	10.48±0.90	12.65±0.34
*K_3PO_4	25	6.90±0.55	10.86±0.10	7.16±0.33	11.05±0.28	7.03±0.39	10.95±0.19
	50	6.21±0.30	8.89±0.25	6.98±0.42	8.95±0.52	6.59±0.14	8.92±0.18
	100	4.39±0.90	5.48±0.27	4.18±0.23	6.00±0.25	4.28±0.55	5.74±0.03
*$Ca(OH)_2$	25	15.34±0.79	21.27±0.69	15.01±0.30	20.10±0.28	15.17±0.36	20.68±0.48
	50	10.72±1.54	16.51±0.72	10.25±0.04	17.00±0.39	10.48±0.78	16.75±0.17
	100	10.03±0.60	10.54±0.49	11.16±0.30	12.08±0.10	10.59±0.16	11.31±0.29
*$CaCO_3$	25	18.46±1.25	24.13±1.15	17.99±0.18	23.85±0.21	18.22±0.70	23.99±0.55
	50	14.68±0.62	21.33±0.52	15.00±0.25	21.75±0.60	14.84±0.23	21.54±0.07
	100	11.65±0.73	13.82±0.58	11.35±0.56	14.09±0.22	11.50±0.37	13.95±0.28
Penconazole	300	8.49±0.78	7.90±0.49	8.11±0.27	6.98±0.36	8.30±0.44	7.44±0.42
	400	5.25±0.96	4.79±0.66	5.76±0.31	4.48±0.47	5.50±0.41	4.63±0.56
	500	3.29±0.79	3.68±1.06	3.00±0.04	3.85±0.25	3.14±0.40	3.76±0.53
Control	-	35.24±0.52	35.47±0.12	35.82±0.25	36.01±0.19	35.53±0.48	35.74±0.25
Mean		12.69	14.83	12.74	14.71	12.71	14.77

BI: Before inoculation; AI: After inoculation

$CD_{(P = 0.05)}$	2009	2010	Pooled		2009	2010	Pooled
BI/AI	0.28	0.11	0.15	Chemical x Concentration x BI/AI	1.50	0.58	0.81

The application of SAR activators before pathogen inoculation showed significantly lower disease intensity (12.69%) in comparison to 14.83% in SAR application after pathogen inoculation (Table 3). The per cent disease control recorded by each SAR activator before inoculation proved superior to after inoculation (Figure 1b). The plants utilize their own defense mechanism for restriction of pathogen development. As markers of resistance, physiological changes always appear in certain intervals after application of the biotic and abiotic inducers against pathogens (Schonbeck et al., 1993). As in application of SAR activators before pathogen inoculation, these physiological changes (markers of resistance) occur before the pathogen inoculation. In SAR activators application after pathogen inoculation these changes occur after pathogen infection. This may be the reason that application of SAR activators before inoculation proved superior to the application after inoculation. Our observations are in accordance with the findings of numerous workers (Schneider et al., 1996; Kuc, 2001; Bokshi et al., 2003; Gozzo, 2003; Soylu et al., 2003). However penconazole was statistically at par in both the cases of application, because of its systemic activity and direct inhibition of the pathogen.

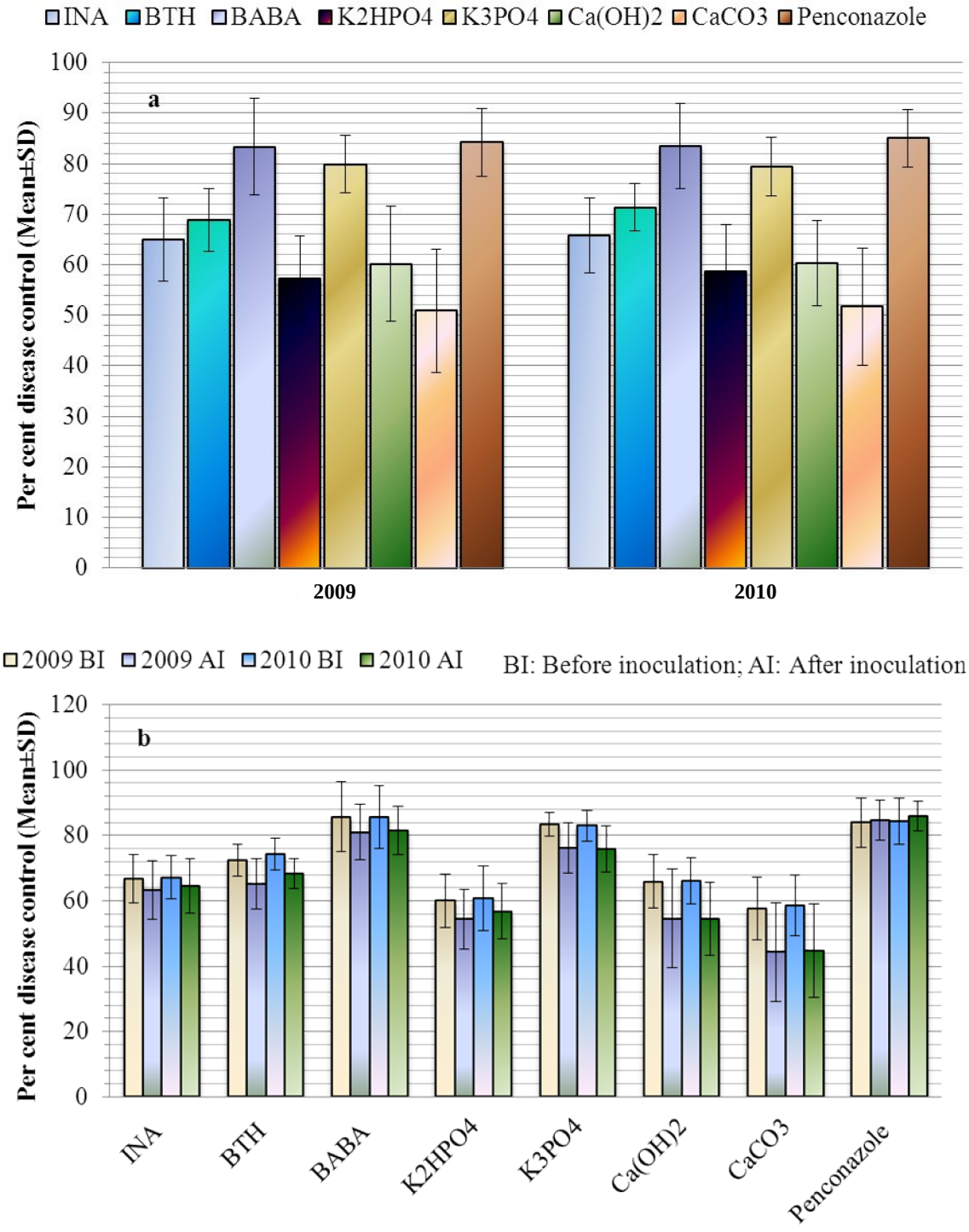

Figure 1. Per cent disease control of Alternaria leaf blotch of apple by SAR activators

In present study application of BABA before and after inoculation of the *Alternaria mali* proved superior to all other SAR activators tested. Similar observations have been recorded by Amzalek and Cohen (2007) who found BABA significantly better than BTH and INA against sunflower rust. The post infection inhibitory effect of BABA better than other SAR activators is because the BABA operates quite rapidly to stop growth of mycelia in the mesophyll as reported by Amzalek and Cohen (2007).

All the three concentrations of each chemical significantly differed from each other with the least disease intensity observed in highest concentration of each chemical.

Conclusion

Alternaria mali isolates exhibited considerable variation in their virulence. The wide variation of isolates indicated that the fungus has a high potential to adapt to resistant cultivars or fungicides. The evidence of random distribution of variation in pathogen population has practical implications for breeding programs as well as in the management of Alternaria leaf blotch. Since the pathogen cannot adapt to SAR chemicals and there is not any such report, so the SAR chemicals were evaluated against the pathogen. All the SAR chemicals except $Ca(OH)_2$ and $CaCO_3$ have been evaluated for the first time against *Alternaria mali*. SAR compounds, especially BABA were effective in managing the disease. This induced resistance exploiting natural defense machinery of plants could be proposed as an alternative, non-conventional and eco-friendly approach for plant protection.

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PLANT SCIENCE

Gall-induced stress in the leaves of *Terminalia arjuna*, food plant of tropical tasar silkworm, *Antheraea mylitta*

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Abstract

Tasar silkworm *Antheraea mylitta* Drury is reared on one of the important food plants, *Terminalia arjuna* (Arjun). Leaf gall Trioza fletcheri minor (Hemiptera: Psyllidae) is the most important gall forming insect on the leaves which has bearing on productive traits of tasar silkworm. Gall formation is the consequence of interaction between the offensive stimulus of the insect and the defensive response of the plant. Hence, the stress on Arjun leaves imparted due to gall formation was studied. There was significant decrease in photosynthesis rate ($P<0.001$), transpiration rate ($P<0.05$) and stomatal conductance ($P<0.05$) in gall infested leaves in comparison to healthy ones while no change in leaf temperature. The oxidative stress assayed through lipid peroxidation and hydrogen peroxide production was found to be significantly higher ($P<0.001$) in gall infested leaves than that of healthy ones. Non-enzymatic anti-oxidant, ascorbic acid content was found to be high in gall leaves while there was decrease in reduced glutathione content ($P<0.01$). The total protein and moisture content values were recorded to be higher in gall infected leaves ($P<0.01$) than the healthy ones indicating towards nutrient flux for the gall insect.

Key words: *Antheraea mylitta*, Gall, Oxidative stress, Photosynthesis rate, *Terminalia arjuna*

Introduction

Commercially important tropical tasar silkworm *Antheraea mylitta* Drury is reared outdoor on *Terminalia tomentosa* (Asan) *and T. arjuna* (Arjun). Wild tasar silkworm populations are also found in forests in tropical India on *Shorea robusta* (Sal), Asan and some other secondary plants. All these primary and secondary food plants are attacked by large number of insect pests. Leaf gall *Trioza fletcheri* minor (Hemiptera: Psyllidae) is the most important gall forming insect on the leaves of primary tasar food plant (Singh and Thangavelu, 1994). A major part of the life cycle of gall insect is closely associated with the formation of gall guild in which the insect almost completes its development by deriving its nutrition from the gall tissues.

Gall formation is the consequence of interaction between the offensive stimulus of the insect and the defensive response of the plant (Raman, 2007). Since insects derive their nutrition from gall tissue, the gall becomes a sink for different nutrients and energy that will be vital for gall insect's growth. The induction and development of galls expose plant tissues to high oxidative stress (Schonrogge et al., 2000; de Oliveira and Isaias, 2010; de Oliveira et al., 2010). Plants have several scavenging mechanisms to limit the accumulation of harmful amounts of active oxygen species (Chaman et al., 2001; Ni et al., 2001). The antioxidants such as glutathione, ascorbic acid, etc. scavenge reactive oxygen radicals, and they are substrates for the antioxidant enzymes. Development of gall has a bearing on the photosynthetic activity of the plant. The attack of gall inducers may cause either negative (Gailite et al., 2005; Aldea et al., 2006; Fernandes et al., 2010) or positive effects on the photosynthesis of the host organs (Fay et al., 1993; Bogatto et al., 1996). Functioning of the gall involves an effect of mechanical damage to various degrees and/or arthropod-derived chemical signals, both affecting endogenous plant signals and, consequently, physiology of host plants (Raman 2007). Studies on the status of oxidants, antioxidants and photosynthetic efficiency in gall infested leaves of *T. arjuna* is scanty. Thus, the idea was coined to study the stress on Arjun leaves exerted due to gall formation.

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Materials and Methods

Plant material and sampling procedure

Tasar silkworm host plant *Terminalia arjuna* (Arjun) was selected for the study. The period of sampling was 1st week of June when the gall infestation attains the peak. Healthy leaves (fourth leaf from the tip) and counterpart gall infested leaves were used for photosynthesis, transpiration and other parameters. Leaf area and number of galls were counted in all sets of samples so that the variation in gall frequency was at the minimum (data not shown). Leaf samples were immediately stored in -80^{0}C deep freezer for further biochemical analysis. Identical sets of leaf were kept for moisture analysis.

Determination of net photosynthesis rate, transpiration rate, stomatal conductance and leaf temperature

Net photosynthesis rate, transpiration rate, stomatal conductance and leaf temperature were recorded in the healthy and gall infested leaves in the field under bright sunlight condition during the first week of June. These parameters were recorded with handheld photosynthesis system (CI-340) from CID Bioscience (USA). Each observation was repeated thrice for all the leaf samples.

Estimation of lipid peroxidation (LPX)

The level of lipid peroxidation (LPX) was measured in terms of malondialdehyde (MDA), a product of LPX estimated by thiobarbituric acid (TBA) reaction (Heath and Packer, 1968). Both in control and gall infestation, ten leaves each were taken as samples (n=10). Leaf sample (0.5 g) was homogenized in 10 ml of 0.1% (*W*/*V*) trichloroacetic acid (TCA), and the homogenate was centrifuged at 7000 x *g* for 10 min. The supernatant was mixed with 0.5% TBA solution in 20% TCA. The mixture was then heated at 95°C for 45 min and cooled under room temperature. The supernatant was read at 532 nm after removal of any interfering substances by centrifuging at 4000x *g* for 10 min. The amount of thiobarbituric acid reactive substances (TBARS) formed was calculated by using an extinction coefficient of $1.56x10^5$ M^{-1} cm^{-1} (Wills 1969), and expressed as nmol TBARS/g weight (wt) tissue.

Estimation of hydrogen peroxide (H2O2)

Hydrogen peroxide content was determined according to the method of Sergiev et al. (1997). Both in control and gall infestation, ten leaves each were taken as samples (n=10). Leaf sample (0.5 g) was homogenized with 10 ml of 0.1% (*W*/*V*) TCA in an ice bath. The homogenate was centrifuged at 7000 x *g* for 10 min, and the supernatant was added with 50 mM potassium phosphate buffer (pH 7.0) and 1 M potassium iodide (KI). The absorbance was read at 390 nm. H_2O_2 was used as standard and expressed as nmol H_2O_2/g wt tissue.

Estimation of ascorbic acid, reduced glutathione and total proteins

For ascorbic acid assay, plant material was homogenized in an ice bath with 10 ml of 0.1% (*W*/*V*) TCA and centrifuged at 7000 x *g* for 10 min. The deproteinised supernatant was used as assay for ascorbic acid following the stoichiometric reduction of phosphomolybdate by ascorbic acid (Mitusi and Ohata, 1961). Ascorbic acid was used as the standard, and results were expressed as µg AsA/g wt tissue. Reduced glutathione content was estimated using Ellman's (DTNB) reagent (Ellman, 1959). Aliquots of supernatant were mixed with 0.6 mM DTNB and incubated for 30 min at room temperature in dark. The sample was finally centrifuged at 2,000 x g for 10 min at room temperature and the absorbance of the supernatant was measured at 412 nm. GSH was taken as standard and expressed as µmol GSH/g tissue. Protein concentration of samples was estimated according to the method of Lowry et al. (1951).

Statistical analysis

Data were analyzed for mean, standard error of mean (SEM), coefficient of variation (CV) and Student's t test using SPSS-11 (SPSS-Inc, Chicago, USA).

Results

For the comparison of healthy and gall infested leaves, *T. arjuna* plants in the economic plantation of field laboratory, CTR&TI, Ranchi were selected. Care was taken to select the position of leaves on the twig where one side was healthy and the other side was infested with gall in order to avoid the variation due to leaf position. Net photosynthesis rate in healthy Arjun leaves was found to be significantly more ($P < 0.001$) than that of gall infected leaves (Table 1). The coefficient of variation (CV) was found to be identical. Transpiration rate and stomatal conductance also showed the similar trend. Transpiration rate was significantly high ($P < 0.05$) in healthy leaves than the gall infected leaves (Table 1). The stomatal conductance also recorded to be high ($P < 0.05$) in healthy leaves in comparison to the gall infested leaves (Table 1). However, no significant difference was observed for the leaf temperature in both types of leaves (Table 1).

Table 1. Net photosynthesis, transpiration rate, stomatal conductance and leaf temperature of healthy and gall infected Arjun leaves.

Parameters	Status	Mean± SEM	Coefficient of variation (CV)	t value	p value
Net photosynthesis rate (µmol/m^2/sec)	H	11.31 ± 0.469	13.825	4.679**	0.001
	G	8.535 ± 0.311	12.132		
Transpiration rate (µmol/m^2/sec)	H	2.41 ± 0.114	15.794	2.359*	0.015
	G	2.02 ± 0.108	17.742		
Stomatal conductance (nmol/m^2/sec)	H	149.3 ± 8.642	19.292	1.831*	0.041
	G	126.85 ± 7.787	20.461		
Leaf temperature (^{0}C)	H	31.262 ± 0.147	1.566	1.751	0.098
	G	30.915 ± 0.117	1.262		

H – Healthy, G – Gall infested *P < 0.05, **P < 0.01

Insect herbivory of *T. arjuna* by *Trioza fletcheri* minor exerted a significant increase in lipid peroxidation (P < 0.05), measured as malondialdehyde, as compared to the healthy ones (Table 2). Infested Arjun leaves recorded higher level of hydrogen peroxide than the control healthy leaves which was statistically highly significant (P <0.001) (Table 2). The coefficient of variation among data set was also comparable. Infested Arjun leaves had higher content of ascorbic acid as compared with their respective controls (Table 2). Stressed Arjun leaves showed significantly less (P < 0.001) reduced glutathione (GSH) in comparison to healthy counterpart (Table 2). Protein content in gall infested Arjun leaves was found to be significantly more (P < 0.001) in comparison to the healthy ones (Table 2). Similar type of observation was also recorded for the moisture content of leaf where the difference in the mean was statistically significant (P <0.01) (Table 2).

Discussion

Net photosynthesis rate in healthy Arjun leaves was found to be significantly more than that of gall infected leaves. Transpiration rate and stomatal conductance also showed similar trend. This may be due to the reduction of net chlorophyllous area of leaf due to gall formation, besides, higher level of reactive oxygen formation and oxidative damages of cell membranes. However, no significant difference was observed for the leaf temperature in both types of leaves. Recent studies have shown lower photosynthetic rate (de Oliveira et al., 2011) and pigment contents in galls (Yang et al., 2003) which support our findings. In other studies, deficiency of several pigment-protein complexes of the light-harvesting complex of photosystem II in gall tissues could account for decreased photosynthetic function at the level of light-harvesting, energy transfer and photochemical energy conversion (Yang et al. 2007; Huang et al. 2009).

Table 2. Oxidative stress related and other parameters in healthy and gall infected Arjun leaves.

Parameters	Status	Mean± SEM	Coefficient of variation (CV)	t value	p value
Lipid peroxidation (LPX) nmol malondialdehyde (MDA)/g fresh tissue	H	151.42 ± 2.94	28.483	2.174*	0.022
	G	188.79 ± 9.93	17.523		
Hydrogen peroxide (H_2O_2) nmol /g fresh tissue	H	120.16 ± 4.61	12.789	3.528**	0.001
	G	149.52 ± 6.41	14.289		
Ascorbic acid content µg/g fresh tissue	H	305.33 ± 36.75	40.116	0.645	0.263
	G	343.03 ± 41.51	40.334		
Reduced glutathione (GSH) µmol/g fresh tissue	H	79.61 ± 1.231	5.153	7.349**	0.001
	G	62.51 ± 1.833	9.777		
Protein content mg/g fresh tissue	H	23.086 ± 1.248	18.011	3.814**	0.001
	G	29.428 ± 0.966	10.938		
Moisture content (%)	H	42.457 ± 1.474	11.573	2.634	0.01
	G	48.634 ± 1.515	10.381		

H – Healthy, G – Gall infested *P < 0.05, **P < 0.01

It has been shown that insect herbivory induces oxidative responses in plants (Chaman et al., 2001; Ni et al., 2001). Plants have several scavenging mechanisms to limit the accumulation of harmful amounts of reactive oxygen species. The antioxidants such as glutathione, ascorbic acid, phenolics, carotenoids and tannins scavenge reactive oxygen radicals, and they are substrates for the antioxidant enzymes (Valko et al., 2007). Insect herbivory of *T. arjuna* by *Trioza fletcheri* minor exerted a significant increase in lipid peroxidation, measured as malondialdehyde, compared with healthy ones. Biotic and a biotic stresses stimulate the production of active oxygen and subsequently lipid peroxidation of the cell macromolecules (Baker and Orlandi, 1996). The increase in lipid peroxidation may be due to the un-capability of antioxidants to capture all the reactive oxygen species produced by this biotic stress. Oxidation of unsaturated fatty acids by singlet oxygen produces distinctly different products such as MDA (Devasagayam et al., 2003). In the present study, MDA content as an expression of lipid peroxidation was increased by gall infestation.

Infested Arjun leaves had higher level of hydrogen peroxide than the control healthy leaves. According to Rosseti and Bonatti (2001) and Zentgraf (2007) one of the early events activated by the hypersensitive response (HR) is the production of ROS, including hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-). Stressed Arjun leaves showed significantly less reduced glutathione (GSH) in comparison to healthy counterpart. Ascorbate and glutathione play essential roles in plant metabolism and stress tolerance. Glutathione is a water soluble antioxidant which reacts directly or indirectly with the reactive oxygen species so, reduces stress injurious effects on membrane. Glutathione play an important role in the protection against oxidative stress. It is involved in the ascorbate/ glutathione cycle and in the regulation of protein thiol–disulphide redox status of plants in response to abiotic and biotic stress (Mullineaux and Rausch, 2005). The reduced glutathione content in the present study indicates their utilization during stress condition.

In the present study ASA level was found to be higher in gall infested leaves (Table 2). In contract to ASA, reduced glutathione level significantly decreased in gall infested leaves when compared to healthy ones. In plants, ascorbic acid and glutathione are two important free radical scavengers and their formation is interrelated (Noctor and Foyer, 1998). Enhancement of H_2O_2 in the present study might have resulted in induction of ASA synthesis and their conversion to protect cells against oxidative damage. Similar observations were made by Khattab and Khattab (2005) in Eucalyptus trees infested with gall.

Protein content in gall infested Arjun leaves was significantly more. Similar type of observation was also recorded for the moisture content of leaf. Singla and Grover (1994) recorded that the rate of protein synthesis declines during stress condition. The increase in protein may be due to the fact that the gall acts as a nutrient sink for the gall insects to develop. In our study the leaf sample was prepared including the gall and the insect residing in it. Dorchin et al. (2006) reported the photosynthesis and sink activity of wasp-induced galls in *Acacia pycnantha* where they found substantial increase in the availability of plant resources for the development of wasps in galls. So for the growth and metabolism, the protein flux might have been channelized to gall area. High moisture content also supports this hypothesis. However, during the process of leaf maturation, the galls fall apart and there is the chance of loss of protein content in the leaves, which needs further studies. Further studies on carbohydrate metabolism are required as there is indication of photosynthesis stress in gall infested leaves.

Conclusions

The study has come out with the finding that, due to gall infestation, the Arjun leaves face different types of stress. There is reduction in photosynthesis rate, respiration rate and stomatal conductance. Oxidative stress also increases as expressed through higher content of hydrogen peroxide. Higher content of lipid peroxidation indicates the damaging effects of ROS produced by gall infested plants. Furthermore, less glutathione indicates their utilization in scavenging action for free radicals. For completion of life cycle of the gall insect, there is a flux of protein content associated with increase in moisture. However, as a whole the leaf comes under stress condition.

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PLANT SCIENCE

Evaluating the grafting approaches for utilizing the rice straw as environmental friendly and potential low cost hydrogels

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Abstract

The present work deals with producing water absorbent hydrogels from rice straw (RS) without any pretreatment. The utilization of RS (an undesirable bio waste) for production of the required hydrogels will add highly to the economic value, contribution reducing the environmental impact of waste disposal and most importantly provide a potentially inexpensive alternative biopolymer to the existing commercial hydrogels from petrochemical origins. The required hydrogels were prepared on a Lab- and Pilot scale via different graft copolymerization approaches with acrylonitrile, by using a technique which reduces the environmental impact, resulting from loading of waste-water by non-reacted monomer, to a minimum. The prepared hydrogels were characterized by using chemical analysis, IR- spectra and thermo gravimetric analysis TGA analysis. As well as, the role of grafting approach and pH-value on affinity of the hydrogels produced to absorb water (distilled and Nile water) was evaluated. The results obtained show that, grafting of precyanoethylated RS, using persulfate-persulfite initiation system, followed by alkali hydrolysis provide hydrogel with relatively higher absorption capacity towards both distilled and Nile water than using Ferrous sulfate-H_2O_2 initiation system. The candidate hydrogels have no toxic (inhibitory) effect against the most efficient micro-organisms in plants media (rhizosphere).

Key words: Hydrogels, Agricultural waste management, Rice straw, Eco-grafting approach, Water absorbent biopolymer, Biopolymer application

Introduction

Sandy soils are wide spread in Egypt and other countries of the Middle East. They cover vast areas that can help in solving the shortage in food production. Incorporating highly water-absorbent polymers (hydrogels), in sandy soil is one technique used as soil conditioners.

Highly water-absorbent materials were first discovered by a group at the North Regional Research Laboratories in the course of an application study of graft copolymers of starch (Burr et al., 1976; Fanta et al., 1984). Presently, there is more than one method for the preparation of hydrogels such as: (a) Hydrogels from industrial polymers [e.g., the preparation of β-acryloxypropionic acid from the chemical initiating the monomer of ethylene glycoldimethacylate (Ansel, 1995). (b) Hydrogels from conventional chemical modification of rice hulls by concentrated nitric acid or phosphorylation of cellulosic material (Saito et al., 1991; Buchholz and Peppas, 1994; Saito et al., 1995), and (c) Hydrogels from graft copolymerization of starch, cellulose, by-products hemicelluloses and lignin, as well as cellulose and chitosan derivatives with vinyl monomers containing a carboxyl, sulfonic, or nitrile group, in the presence of cross-linking agents. In the case of using acrylonitrile as a vinyl monomer, the alkali hydrolysis must be carried out on the prepared grafted samples (Kennedy et al., 1993; Athawale and Lele, 2000; Li and Pan 2010; Singh et al., 2012). Copolymerization of acrylamidomethyl propane sulfonic acid and acrylonitrile onto starch resulted in hydrogel materials without needing an alkali hydrolysis stage.

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In 1990, in the framework of cooperation between the International Trade Ministry and the Industrial Ministry of Japan, the Japanese Aids Programe used highly water-absorbent product as a soil conditioner for sandy soil in Egypt. However, this experiment had failed because of unfixation of hydrogels toward light.

In the previous investigations (Fanta and Doane, 1990; El-Saied et al., 2000; Kume et al., 2002), hydroxyethyl cellulose (HEC) and other polysaccharides (i.e., konja mannan, alginic acid, agar, pectin, starch and chitin) were selected as the trunk polymers, these polysaccharides were prepared as graft copolymers with partially hydrolyzed polyacrylamide (PAM) or polymethyl acrylate (PMA), poly-(2-dimethylamino) ethyl-methacrylate (PDM) or polyacrylic acid sodium and potassium salts (PAANa), to give highly water absorbent- materials. The wastewater from grafting process loads of non-reacted monomer (harmful compound). Synthetic polymer (e.g., polyacrylamide) in gel form are considered as one of the most widely used materials as soil conditioning for sandy soils, when mixed with such soils (Karlsson, 2000).

Although the extent of application of the synthetic soil conditioner polymers is very wide, especially in desert area, their use in practice is not spread as expected due to high cost. Despite the agricultural wastes represent an inexpensive source of lignocellulosic cellulose products, e.g., artificial wood and paper, most of it caused pollution due to there are good media for growing the microorganisms, or emission of toxic gasses during disposal by burning. The utilization of lignocellulosic wastes, as cellulose source, for economical preparation of hydrogel was studied by numerous authors (Buchholz and Peppas, 1994; Saito et al., 1995). This hydrogel was prepared from chemical treatment of rice hulls by concentrated nitric acid or phosphorylation of cellulosic material.

In Egypt and other similar countries, which have huge amount of agricultural wastes (lignocelluloses), e.g., rice straw, sugar-cane bagasse, cotton stalks etc, normally they use partially in pulp and paper industries, however they are still large amounts, especially RS without any utilization and cause environmental problems. According to the periodical report of Ministry of Agriculture in Egypt for the year 2009, the accumulated quantities of rice straw was about 6 million ton/year.

In our previous work (El-Saied et al., 2000, 2004, 2007), hydrogel was prepared from local endogenous lignocelluloses in pulp form, e.g., sugar-cane bagasse, rice straw and cotton stalks. These hydrogels were prepared via graft copolymerization reactions with some vinyl monomers, using a new technique which reduces environmental impact, resulting from unreacted monomer, to a minimum. The pore size distribution and its relation with moisture retention capacity (as a preliminary examination) of sandy soil loaded with hydrogel-based lignocellulosic materials recommended the utilization of such material as soil conditioner. So it is aimed to evaluate in detail, the utilization of these foregoing hydrogels as soil conditioner in sandy soil, comparing to other conventional soil conditioners (organic fertilizer and bentonite). Despite the success of this technology to overcome the agronomic obstacle of sandy soil, their use in practice has not been recommended due to it needs pretreatment to reduce the noncellulosic constituents, which add cost during production as well as environmental impact from disposal of treatment liquors.

The present work deals with producing hydrogels from raw rice straw (RS) without any pretreatment. The role of grafting approach and type of acid used to neutralize the gel, on affinity of the hydrogel produced to absorb water (distilled and Nile water) was evaluated. In forthcoming work we will be concerned on evaluating these RS-based hydrogels as preserving agents for fertilizers in sandy soil, and as adsorbents for utilization of industrial waste water in irrigation purpose. The safe use of the investigated hydrogels as soil conditioners was also evaluated from examining their toxicity behavior against the most efficient micro-organisms in plants media (*rizosphere*).

Experimental

Grafting of rice straw in lab scale

In this work grafting of RS with acrylonitrile monomer, using radical intermediate were selected. The following initiation systems were applied;

Grafting via Hydroxyl Radical Initiation

Based on our previous work (El-Saied et al., 2007), ferrous ammonium sulfate- hydrogen peroxide-thiourea dioxide system, was used, as such system is regarded as the most suitable initiator onto carbohydrate. The graft copolymerization was carried out through a two-stage process. In the first stage the grinned RS (2-3 cm), without any purification, was impregnated with 0.1% ferrous ammonium sulfate, in a liquor ratio of 20 (wt/vol), for 15 min. at room temperature(~ 30°C). Then it was filtered under suction and pressed to remove the excess ferrous

ammonium sulfate. For the second stage, the desired amount of H_2O_2 (0.12 of 30% soln. /based on RS), and thiourea dioxiode (equivalent the amount of H_2O_2, wt/wt) were added to the treated lignocellulose (RS). The mixture is adjusted to pH 6.5 followed by adding different ratios of monomer acrylonitrile (AN) with a few drops of emulsified soap and completing the solution to a liquor ratio of 20. The reaction was left to desired time (3 h) and temperature (75°C)**.** The grafted samples were filtered, washed several time with water until neutralized and left in a vacuum oven at 65°C to dry. The effects of temperature (50°C instead of 75°C) of grafting and concentration of H_2O_2 (0.02-0.16), were also studied.

Grafting via Persulfate Initiation

The grinned raw (RS) were treated with the required amount of AN and emulsifier, followed by addition of the persulfate salt (0.6-3%) with good mixing, and finally added the calculated amount of bisulfate salt (0.3-1.5%) with continuing the mixing for the required time (3 h) at 45°C. The prepared copolymers are filtered, washed several times with water until neutralized and dried in a vacuum oven at 65°C.

The efficiency of grafting of vinyl monomer to lignocellulose using foregoing initiating systems are determined by determination of polymer load and homopolymers, from nitrogen content, using Kjehldal method, before and after soxhelet extraction for 48 h by dimethylformamide.

Preparing Hydrogels on a Pilot Scale

In this study the preparation of hydrogels were carried out through the following two steps:

Grafting Copolymerization for synthesis polyacrylonitrile-grafted-rice straw; PAN-g-RS (first step)

The promising grafting efficiencies of previous mentioned grafting processes were used to produce hydrolyzed grafted copolymer (HPAN-g-RS) on a pilot-scale. In both processes air dry milled (RS) was used (~1.5Kg) and mixed with AN (~3Kg) in presence of different initiators. About 4.5 Kg of the produced hydrolyzed copolymers are obtained. AN was grafted by using hydroxyl radicals (Fe2+ / H_2O_2 and persulfate initiator) according to the selected conditions which were provide higher monomer conversion;

- $0.16H_2O_2$ (30%), 2AN, both based on oven dryweight of RS and liquor ratio 20, at temp. 50^{o}C for .3 h), and
- 2.4% persulfate, 1.2 % bisulfite, 2 AN/RS, and liquor ratio 20, at temp. 50^{o}C for .3 h)

The grafting step was carried out in stationary-tank mixture of capacity 60 l, with a driving motor of 1.6 hp, connected with a gearbox to stir at 23 rpm/min.

For the trial to enhance the persulfate-persulfite initiation system to produce high grafted RS, cyanoethylation of RS was carried out as pre-activation step. In this process RS was treated by 5% NaOH (based on RS), and 10% from the total amount of AN used in grafting, for 1 h, at room temperature (~35 ^{o}C), followed by adjusting the pH to 6-7. The grafting process was carried out under the same conditions of persulfate-persulfite initiation system, but by using the remained per cent of AN (90%).

Alkali Hydrolysis of Polymerized Rice Straw (second step)

The above prepared PAN-g- RS were subjected to alkali hydrolysis by KOH, to improve its absorbent properties. The experimental conditions of hydrolysis are 0.6 mole KOH per mol of AN, which is used in grafting, a 30 liquor ratio, and a temperature between 90^{o}C and 100^{o}C for 2h. The excess alkali was neutralized with dilute mineral and organic acids.

Characterization of PAN-g- RS and hydrogels

PAN-g-RS and HPAN-g-RS products were characterized by the following techniques:

Fourier transform infrared (FTIR)-spectra measurement

IR-spectra (4000-400 cm^{-1}) were recorded on Nexus 670 FTIR spectrophotometer (Iclet Co., USA), using KBr disc. The technique of O'Connor et al (O'Connor et al., 1958) was used to calculate the crystallinity index (Cr.I). The mean strength of hydrogen bonds (MHBS) was calculated according to Levdik et al. (1967).

Thermal analysis

Thermal gravimetric (TGA) and derivative of thermaogravimetric (DTG) analyses of the examined samples were done using a PERKIN ELIMER Thermogravimetric Analyzer (TGA 7). The analyses were performed with a heating rate of 10^{o}C/min and a flow rate of 50 ml /minute, under non-isothermal conditions and in the presence of nitrogen.

TG-curve analysis

Kinetic studies, based on the weight loss data, were obtained by TG curve analysis. The activation energy has been evaluated by applying Coat and Redfern (1964), method of analysis. For pseudo homogeneous kinetics, the irreversible rate of

conversion of the weight fraction of reactant was expressed by the following equation:

$$\frac{d\alpha}{dt} = k(1-\alpha)^n \qquad (1)$$

Where, α is the fraction of material decomposed at time t, k is the specific rate constant and n is the order of reaction. The temperature dependence of k is expressed by the Arrhenius equation:

$$k = A\, e^{-Ea/RT} \qquad (2)$$

Where A is the frequency factor (s^{-1}) and T is the absolute temperature.

For linear heating rate, a, (deg.min.$^{-1}$):

$$a = \frac{dT}{dt} \qquad (3)$$

For calculating the activation energy, Ea, of thermal decomposition when n = 1, equation 4 was used.

$$\log\left[-\log\frac{1-\alpha}{T^2}\right] = \log\frac{AR}{aE_a}\left[1-\frac{2RT}{E_a}\right] - \frac{E_a}{2.3RT} \qquad (4)$$

When $n \neq 1$, equation 5 was used;

$$\log\left[\frac{1-(1-\alpha)^{1-n}}{T^2(1-n)}\right] = \log\frac{AR}{aE_a}\left[1-\frac{2RT}{E_a}\right] - \frac{E_a}{2.3RT} \qquad (5)$$

Plotting the left-hand-side value of the equation { i.e., log [1- $(1-\alpha)^{1-n} / T^2(1-n)$]} against 1 / T using various values of "n" should give a straight line with the most appropriate value of "n" (Basta, 1999). The Least square method was applied for the equation, using values of "n" ranging from 0.0 to 3.0 in increments of 0.5. The correlation coefficient (r) and the standard error (SE) were calculated for each value of "n". The "n" value, which corresponds to the maximum r and minimum SE, is the order of the degradation process. The activation energies and frequency factors were calculated from the slope and intercept, respectively, of the Coat-Redfern equation with the most appropriate value of "n".

Water absorption capacity

In this study certain weight of the hydrogel produced was set in sufficient amount of distilled water or Nile water for 24 hours. Excess water over the swollen samples was weighed (W_1), then dry in oven at 105°C and weighed again (W_2).

$$\text{Water absorption} = \frac{W_2 - W_1}{W_1} \times 100$$

Toxicity (inhibitory) test against the most efficient micro-organisms in plants media (rhizosphere)

This test was carried out according to the following steps (Abdel-Hameed et al., 2008):

- In 100 ml flasks, each medium was inoculated with the proper microorganism and incubated at 28-30°C for 7 days for total microbial count and 15 days for the other microorganisms.
- After incubation period, 5 ml from each inoculated medium were diluted to 400 ml using the same medium (but not inoculated with the micro -organism) and was poured into sterile Petri dishes (10 ml/ each dish).
- To examine the degree of toxicity (the anti - microbial activity) for the prepared hydrogels on examined microbes, four small blocks of the solidified medium were cut out from the plates by means of sterile 0.5 cm cork borer and removed with a sterile needle. In the well-formed, a piece of each hydrogel (100 mg each) was placed. The dishes were incubated at the degree of 28-30°C for the proper time (15 days). The same experiment was triplicated. The anti-microbial activities were expressed as the width of zones of inhibition in millimeters. The affected area in each dish was counted and the mean of replicates was recorded (Abdel-Hameed et al., 2008).

Results and Discussion

Table 1 indicates the average values of the chemical compositions obtained from analyzing different types of available rice straw (RS). Chemical analyses were determined by standard methods (Markblatt IV/29, Jayme and Sarten, 1940; The Institute of Paper Chemistry, 1951).

Table 1. Chemical composition of rice straw.

Analysis	Mean value ±SD
Extractive(MeOH/Benzene)	3.05±0.141
Ash	16.60±0.622
Lignin	12.01±0.157
Pentosans	21.70± 0.481
Hollocellulose (free ash)	55.01±1.532

Effect of grafting approach (in Lab. Scale)

The polymer load and the monomer conversion percentages were recorded in Tables 2-4. For grafting via hydroxyl radical initiation, using Fe^{+2}-H_2O_2 system, Table 2 shows that, increasing the AN/RS ratio from 0.5 to 2 accompanied by

increasing the polymer loading from 7.5% to ~61%, and there is no increase in the percent of grafting with further increase this ratio than 2. As can be noticed that, lowering the temperature of graft copolymerization from 75°C to 50°C has a profound effect (increased) the polymer loading of RS; whereas the polymer loading increased from ~ 61% to 85.504%.

On studying the effect of H_2O_2 concentration on polymer load percentage, under the foregoing optimum temperature and AN/RS ratio, Table 3 shows that at relatively high H_2O_2 concentration 0.16% based on RS optimum polymer load (92.39%) and monomer conversion (46.193%) percentages are obtained. The explanation of this observation depends on the following mechanism (Bains, 1972; Kolthoff and Barney, 1951);

- When Fe^{2+} ions are mixed with H_2O_2, $HO^{\cdot}$ radicals are generated.

$$H_2O_2 + Fe^{2+} \longrightarrow HO^- + HO^{\cdot} + Fe^{3+},$$

$$HO^{\cdot} + Fe^{3+} \longrightarrow HO^- + Fe^{2+}$$

- The $HO^{\cdot}$ radicals further decompose H_2O_2

$$HO^{\cdot} + H_2O_2 \longrightarrow H_2O + HO^{\cdot}_2,$$

$$HO^{\cdot}_2 + H_2O_2 \longrightarrow HO^{\cdot} + H_2O + O_2$$

The formed hydroxyl radical initiate both the grafting reaction between AN and RS with the formation of PAN-g-RS, or polymerization of AN with the formation of PAN (homopolymer). The decomposition of H_2O_2 by the hydroxyl radicals takes place only if H_2O present in large excess over Fe^{+2} ions (Kolthoff and Barney, 1951).

For the second grafting initiation system (per sulfate-bisulfite) in comparison with ferrous-hydrogen peroxide system, Tables 2-4 show that, the maximum polymer loading and AN conversion percentages were obtained on using 2.4% persulfate. This value is higher (149.385) than that obtained by Fe^{2+}-H_2O_2 initiation system (92.386). The change in persulfate is more significant on these grafting parameters than the case of Fe^{+2}-H_2O_2 system.

Table 2. Effect of Acrylonitrile to Rice straw ratio on grafting efficiency.

Exp.no.	AN/RS	Nitrogen %±SD	Polymer loading, % ± SD	Monomer conversion, % ±SD
1	0.5 : 1	1.83 ± 0.198	7.59 ±0.764	15.19± 2.160
2	1 : 1	2.88 ± 0.265	12.50 ± 1.022	12.5 ±1.445
3	2 : 1	9.81 ±0.495	60.87 ± 1.91	30.435±2.699
4	3 : 1	7.70 ±0.485	42.24 ± 1.870	14.08 ±2.646
5*	2: 1	11.95±0.306	85.504 ± 1.180	42.752 ±1.689

N.B: Liquor ratio is 20 : 1, H2O2 : 0.12, for 3h at 750C.

* the same Expt. conditions but at temp. 50oC

Table 3. Effect of H_2O_2 concentration on grafting efficiency.

Expt.no.	H_2O_2 (30%) / RS	Nitrogen, % ±SD	Polymer loading,% ±SD	Monomer conversion, % ±SD
6	0.02	8.9±0.39	52.27±1.504	26.135 ±2.127
7	0.04	11.56±0.405	80.468±1.560	40.234 ±1.103
8	0.08	11.19±0.398	75.936±1.535	37.968± 1.919
9	0.12	11.95±0.306	85.504±1.180	42.752 ±1.689
10	0.16	12.45±0.417	92.386± 1.608	46.193± 1.143

N.B. the same expt..conditions as Table 3, but at AN/RS ratio 2/1, and temp. 50oC.

Table 4. Grafting copolymerization of AN onto RS by ammonium persulfate – bisulfite.

Expt. No.	Persulfate, %	Bisulfite, %	Nitrogen % ±SD	Polymer loading, % ±SD	Monomer conversion, %±SD
11	0.6	0.3	12.29 ± 0.354	99.033 ±1.365	49.517 ± 0.965
12	1.2	0.6	14.62 ±0.217	129.313±0.837	64.657 ±0.592
13	1.8	0.9	14.98± 0.308	136.85 ± 1.187	68.425 ±0.389
14	2.4	1.2	15.53 ± 0.401	149.385± 1.549	74.69 ± 1.095
15	3.0	1.5	15.10 ± 0.278	139.48 ± 1.072	69.740 ± 0.758

N.B. Liquor ratio 20:1, AN 2:1, for 3h at 450C .

The priority of persulfate-persulfite system is ascribed to the assumption that, for hydroxyl radicals the depolymerization of cellulose is occurred (Ogiwara and Kubota, 1968), which provide relatively higher free radicals formed on the cellulose backbone, and fast the termination step. While for persulfate-persulfite initiation, the reaction is performed as follows (Kolthoff and Meeham, 1953):

$S_2O_8^{2-} \longrightarrow 2SO^{\cdot-}_4$

$SO^{\cdot-}_4 + \text{Cell-OH} \longrightarrow \text{Cell-O}^{\cdot}$

$SO^{\cdot-}_4 + M \longrightarrow M^{\cdot}$

$\text{Cell-O}^{\cdot} + M^{\cdot} \longrightarrow \text{M-g-Cell}$

$M^{\cdot} + M+. \longrightarrow \text{Homopolymer}$

To evaluate the efficiency and economical application of these grafting systems, the homopolymer-free grafted RS is determined from nitrogen content of DMF-extracted samples obtained under optimum grafting processes. The results obtained are recorded in Table 5. It is noticed that, for two studied processes lower percentages of homopolymers formed, these percentages not exceed 2.30%-3.54%. These promising results and for economical production of hydrogel, the polymerized sample as a whole (grafted sample and homopolymer), was used as the intermediate stage for preparation of hydrogels in large scale.

Effect of grafting approach on hydrogels produced (in Pilot scale)

Table 6 shows the nitrogen content of the prepared hydrogels before and after hydrolyses. The results indicate that, during alkali hydrolysis, the AN groups converted to amide and carboxylic groups (hydrophilic groups.). As can be note that, preactivation by cyanoethylation improves the formation of amide groups, whereas the reduction in nitrogen content in former case is lower than later ones.

Characterization of the prepared grafted lignocellulose and hydrogels:

IR-spectra, thermal analyses and water absorption capacity, were carried out to evidence the grafting of RS as the intermediate step to form amide-containing rice straw as hydrogel material. As well as to examine the effect of initiating system, during grafting process, and pretreating the RS, on the thermal stability of modified RS produced. The examined samples are:

- Raw RS
- PAN-g-RS (1), which was grafted under Expt. conditions # 10,
- PAN-g-RS (2), which was grafted under Expt. conditions # 14
- CE-RS, cyanoethylated RS.
- PAN-g-CE-RS(2), cyanoethylation followed by grafting under expt. Conditions #14.
- HPAN-RS(1), Alkaly hydrolysis of PAN-g-RS(1)
- HPAN-g-RS(2), alkali hydrolysis of PAN-g-RS(2)
- HPAN-g-CE-RS(2), alkali hydrolysis of PAN-g-RS(2)

FTIR-spectra

The IR-spectrum of RS (Table 7), shows characteristic bands at 3433 cm^{-1}, 2925 cm^{-1} due to OH , CH (stretching vibration) and hydrogen bonds (intra & inter molecular hydrogen bonds). The bands at 1727 cm^{-1}, 1637 cm^{-1}, corresponding to C=O (stretchning vibration); while the bands at 1441.5 cm^{-1}, 1373 cm^{-1} and 899.6 cm^{-1} corresponding to OH (bending vib.), CH (bend. Vib.) and CH (rocking vibration), respectively.

Table 5. Grafting efficiency of two initiation systems.

Expt. No.	Grating initiation system	Nitrogen % Unextracted PAN-g-RS ±SD	Extracted PAN-g-RS ±SD	Polymer loading, % ±SD	True grafting, % ±SD	Grafting efficiency,% ±SD
10	Fe^{2+}-H_2O_2 system	12.45±0.417	12.3 ±0.206	92.386 ±1.608	90.2636±0.794	97.702±1.1889
14	Per-sulfate/per-sulfite system	15.53± 0.401	14.42±0.238	149.39± 1.549	146.774±0.918	98.249±0.525

Table 6. Nitrogen content of PAN-g-RS and HPAN-g-RS prepared in pilot scale.

Samples	Nitrogen %		Polymer loading, % ±SD	Reduction in N., %±SD
	Before hydrolysis ±SD	After hydrolysis ±SD		
HPAN-g-RS (1) (Fe^{2+}-H_2O_2 system)	10.39± 0.263	8.3±0.181	66.877 ± 1.0144	19.249± 0.396
HPAN-g-RS (2) (Per-sulfate/per-sulfite system)	15.48 ±0.305	7.11± 0.186	148.1911± 1.176	54.07± 0.4196
HPAN-g-CE-RS(2), Cyanoethylation-grafting using persulfate-persulfite system)	15.51±0.223	8.11±0.1695	148.906± 0.861	47.71± 0.4822

In the IR-spectra of PAN-g-RS (1-2) samples, prepared by using the two foregoing initiating systems, under Expt. conditions # 10 and 14 (Table 7), the band maxima corresponding to stretching vibration of OH were red shifted (from 3433.6m c^{-1} to 3412 cm^{-1}, and 3420 cm^{-1}, respectively), with the appearance of a new band at 2244.7 cm^{-1}, arising from C≅N groups. This is attributed to substituting the hydroxyl groups of glucopyranose units by poly-cyanoethyl group. This group leads to diminish the intra molecular hydrogen bonds between fiber chains, as manifested from decreasing the values of MHBS and crystallinity index. The extent of decreasing MHBS values is related to the efficiency of grafting resulted from changing the initiation system.

The spectra of partially treated (RS) by cyanoethylation before grafting, using persulphate initiator (Table 7), confirm the positive effect of such treatment to improve the ability of (RS) to grafting process. Whereas, the relative absorbance of band corresponds to C≅N groups at 2444.2 cm^{-1} increased from 0.45 to 0.7023, in addition to the blue shift of the band maximum corresponds to OH (str.) from 3433 to 3444 cm^{-1}, and increased its relative absorbance and MHBS values. The reverse trends of increasing the MHBS with decreasing the degree of order (Cr. I) of pre-cyanoethylated (RS) before grafting (PAN-g-CE-RS), this is probable ascribed to the shortest of the formed propagated cyanoethyl group, than PAN-g-RS. Because, the decrystlization of cellulose containing (RS) by cyanoethylation process enhances the number of hydroxyl groups available to block with AN monomer during grafting. It can be also shown that (Figure 5), complete conversion of nitrile group to amide group, on alkali hydrolyses of PAN-g-CE-RS has not been reached, due to the remaining of C≅N band and the relative absorbance of band corresponds to C=O at 1657 cm^{-1} is lower than alkali hydrolyses of PAN-g-RS.

For the IR-measurements of modified RS-samples, Table 7-b shows that the MHBS of hydrolyzed samples are higher than those values of untreated and grafted (RS). This is ascribed to the ability of forming additional inter and intra molecular hydrogen bonding between NH of the amide groups formed with unsubstituted OH groups of glucopyranose units. The appearance of absorption bands at 1556-1564 cm^{-1} indicates the presence of COO^- together with amide groups in the hydrolyzed samples

Thermal stability

The TGA and DTG curves of unmodified RS (Figure 1 and Table 8), show three degradation stages. At lower temperature, i.e. < 112°C (1st degradation stage), the weight loss is due to the evolution of sorbed moisture. The second process, in the range from166-328°C is due to the decomposition of RS components, leading to the formation of carbonaceous char. This is followed by weight loss within the temperature of 328-484°C due to oxidation of charred product (Shafizadeh, 1982). The 2nd and 3rd process regard the main degradation stages.

For PAN-g-RS (1 and 2), the start temperature of the 2nd degradation stages are higher than ungrafted RS. This is due to the blocking of hydroxyl group by poly-AN-groups. As can be noticed that, maximum raise in the start temp. was noticed for PAN-g-RS(1) than PAN-g-RS(2), this is ascribed to the fact that during free radical copolymerization process, the trunk polymer is depolymerized, which leads to scission of chains (Yoshitaka et al., 1968). This effect is more observed at relatively higher grafted RS (PAN-g-RS(2)).

Table 7-a. Main IR-absorption bands and IR-measurements of un- and modified rice straw.
a- Main IR-absorption bands.

Sample	ν_{OH} or ν_{NH} (stretching)		ν_{CH} (stretching)		$\nu_{C\equiv N}$ (stretching)		$\nu_{C=O}$ (stretching)		ν_{OH} or ν_{NH} (bending)		ν_{CH} (rocking)	
	cm^{-1}	E	cm^{-1}	E	cm^{-1}	E	cm^{-1}	E	cm^{-1}	E	cm^{-1}	E
Rice straw	3433.6	0.9923	2925	0.7544	-	-	1727	0.5667	1426	1.1685	896.7	0.4539
							1637.3	0.9372				
PAN-g-RS (1)	3412	1.1279	2925	0.9492	2244.7	0.5915	1724	0.6468	1450	1.143	890	0.2147
							1658.5	0.8998				
PAN-g-RS (2)	3420	0.9584	2926	0.9954	2244.7	0.4597	1659	1.016	1447.3	1.2852	902.5	0.2280
			2880.17	0.7177								
CE-RS	3431	1.3106	2927.4	1.0599	2267.9	0.3551	1665	0.6695	1443.5	0.7275	926.6	0.3221
			2886.9	0.9278			1635.3	0.7326				
PAN-g-CE-RS	3444.2	1.3709	2928.4	1.1547	2247.6	0.70236	1729.8	0.3982	1451.2	0.8641	895.77	0.4307
			2895.6	0.9124			1665.23	0.8739				
							1638.2	0.7815				
HPAN-g-RS (2)	3446.2	3.3858	2928.4	1.4099	-	-	1643.1	1.8345	1432.9	2.452	879.4	0.4667
			2850.3	0.7824			1556.3	1.6299				
HPAN-g-CE-RS (2)	3451.9	2.2021	2929.3	1.1309	2253.4	0.37697	1657.5	1.2434	1460.8	0.8335	874.6	0.3558
			2862.8	0.8099			1564.95	1.2101	1412.6	0.9637		

Table 7-b. IR-Measurements.

Sample	MHBS ($A_{OH(Str.)}$ /$A_{CH\ (Str.)}$)	Cr.I. ($A_{\sim 1370\ cm^{-1}}$ /$A_{\sim 2920\ cm^{-1}}$)
Rice straw	1.3154	1.248
PAN-g-RS (1)	1.1442	1.062
PAN-g-RS (2)	0.96285	1.079
CE-RS	1.2365	0.704
PAN-g-CE-RS	1.1873	0.697
HPAN-g-RS (2)	2.40143	0.774
HPAN-g-CE-RS (2)	1.9472	0.623

Table 8. Kinetic parameters of un- and modified rice straw.

Sample code in chart	stage	Temp. range °C	DTG peak temp. °C	"n"	-r	Se	E_a kJ/ mole	Wt. (final)
Rice straw	1st	50- 112.03	62.57	-	-	-	-	19.045
	2nd	166-327.5	254.07	1.0	0.99498	0.07634	79.365	
	3rd	327.5-484.2	439.65	1.0	0.98895	0.06945	91.948	
							Σ Ea = ١٧١ .٣١٣	
PAN-g-RS (1)	1st	50- 110.1	62.57	-	-	-	-	5.951
	2nd	208.6-273.4	257.4	0.5	0.9811	0.1515	188.4999	
	3rd	351.9- 610	527.4	0.5	0.8974	0.2085	61.674	
							Σ Ea = 250.17	
PAN-g-RS (2)	1st	50- 106	67	-	-	-	-	5.188
	2nd	197.4 –	249.1	1.5	0.9752	0.2009	122.286	
	3rd	358.2	575	1.0	0.9643	0.1605	97.648	
		407.4 - 686.6					Σ Ea = 219.934	
HPAN-g-RS (2)	1st	50- 147.9	85.2	-	-	-	-	12.839
	2nd	249.1 -298	286.3	1.0	0.9477	0.2155	255.19	
	3rd	298.1-340.5	320.6	1.0	0.9594	0.1914	369.16	
	4th	516 -611	564.4	1.5	0.9910	0.1386	508.62	
							Σ Ea = 1132.97	
HPAN-g-CE-RS (2)	1st	50- 100	86.5	-	-	-	-	24.443
	2nd	245.9 -356	338.1	1.0	0.9398	0.241	134.01	
	3rd	356 -387.8	382.3	0.0	0.9599	0.1874	793.05	
	4th	493.2 – 529.9	515	1.5	0.9873	0.1905	1074.89	
							Σ Ea = 2001.95	

TG and DTG curves of alkali-hydrolyzed of grafted samples (HPAN-g-RS and HPAN-g-CN-RS); Figure 2, show increased in onset temperature than grafted RS. This is attributed to the formation of amide and carboxylic groups (NH and C=O groups), which consequently enhances the formation of intra molecular hydrogen bonding with hydroxyl groups, in other glucopyranose units. Therefore, more energy is needed for decomposition (Table 8). This view confirms the increase in MHBS and Cr. I in the case of hydrolyzed samples than unhydrolyzed grafted samples.

Table 8 also shows that, thermal stability of the foregoing examined samples increase in the order:

HPAN-g-CN-RS > PAN-g-RS (2) > PAN-g-RS (1) > PAN-g-RS (3).

Effect of grafting approach on water absorption capacity

This work was carried out as a preliminary study to evaluate the role of grafting process and type of acid used to adjust the pH-value of the produced hydrogels with high water absorption for both distilled and Nile water. The examined hydrogel samples and their water absorption are listed in Table 9.

Table 9 shows that, the increase in the hydrophilic group (COOH) together with the amide groups, as the case of HPAN-g-RS, using persulfate-persulfite initiation system, led to increase its ability to absorbs more quantity of water than HPNA-g-RS Fe(II)-H_2O_2). Also, while cyanoethylation was taken place, some carboxyl groups is formed, this causes to improve the water absorption ability of hydrogel HPAN-g-CE-RS produced.

Generally the water absorption capacities of investigated hydrogels in the case of distilled water are higher than Nile water; this may be ascribed to the high affinity of these hydrogels to bind sodium ions in Nile water, which leads to restrict, to some extent, the swelling behaviour of hydrogels in water. As can be noticed that samples # 1 # 4, # 8 and #10 have the best water absorption property. These samples will be used for the further studies, on evaluation of the prepared hydrogels in agronomic applications under laboratory and green house conditions:

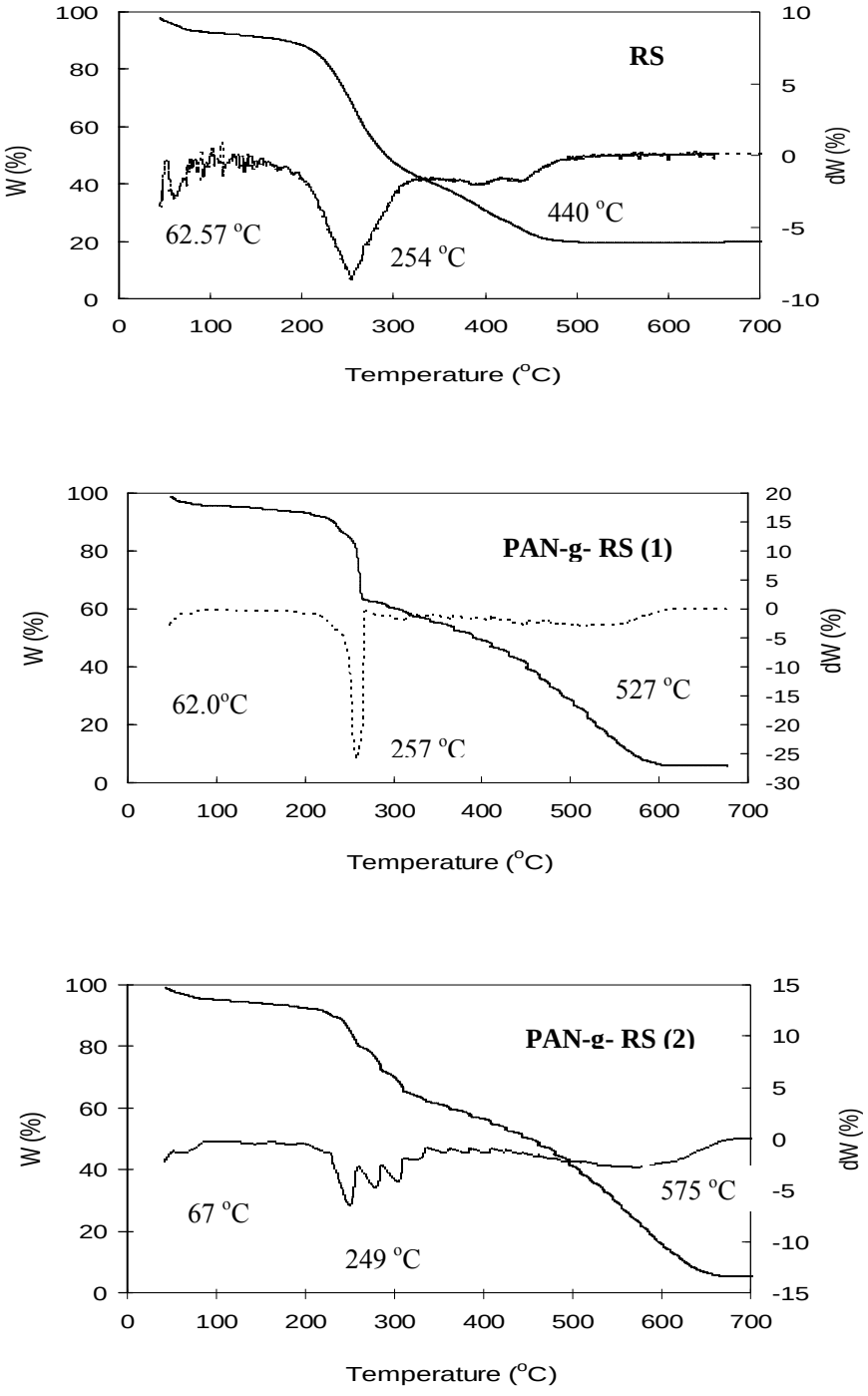

Figure 1. TGA and DTG curves for RS and PAN-grafted RS.

Table 9. Water absorption capacities of hydrogels produced.

Sample No.	Hydrogel	Used acid	Absorption capacity Sat/Dry x١٠٠	
			Distilled water ±SD	Nile Water ±SD
1	HPAN-g-RS (1)	AcOH	6710.4± 17.13	6107.0± 15.556
2	HPAN-g-RS (1)	H_2SO_4	1522.6± 9.637	875.8± 20.082
3	HPAN-g-RS (1)	H_3PO_4	1366.2± 14.425	935.8± 22.345
4	HPAN-g-RS (1)	HNO_3	3318.2± 5.375	2024.7± 20.784
5	HPAN-g-RS (2)	AcOH	2121.9± 18.246	2602.5± 6.364
6	HPAN-g-RS (2)	H_2SO_4	1238.3± 8705	1441.7± 15.981
7	HPAN-g-RS (2)	H_3PO_4	991.9± 10.041	663.9± 8.627
8	HPAN-g-RS (2)	HNO_3	3008.1± 8.414	3128.2± 24.142
9	HPAN-g-CE-RS (2)	H_3PO_4	2403.4± 3.820	1907.1± 21.113
10	HPAN-g-CE-RS (2)	AcOH	7061.4± 11.594	3788.7± 23.517
11	HPAN-g-CE-RS (2)	HNO_3	2794.7± 12.104	1822.1± 14.2846
12	HPAN-g-CE-RS (2)	H_3PO_4 +HNO_3	2166.7± 13.007	2639.5 ± 16.775

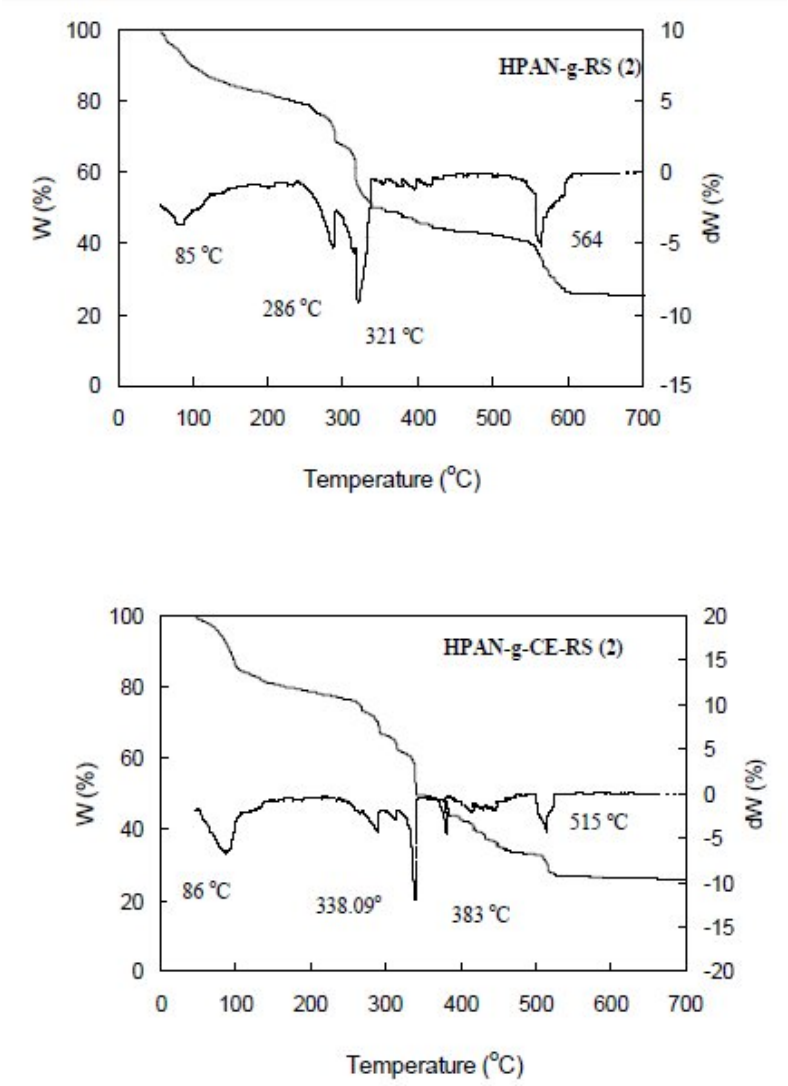

Figure 2. TGA and DTG curves for hydrolyzed PAN-grafted RS.

Table 10. Toxicity test for the effect of the prepared hydrogels on the most beneficent microorganisms in the soil.

Examined hydrogel	*Rhizobia sp*.					*Azotobacter sp.*	*Azospirillum* sp.	*Bacillus suptlis*	Phosphate dissolving bacteria	*Actino-micites*	Fungi
	Alfa-alfa	*Clover*	*Broad bean*	*Peanut (T110)*	*phasoleai*						
Gel #1	--	--	--	--	--	--	---	----	---	--	----
Gel # 2	---	---	---	---	---	---	----	----	----	---	---
Gel # 3	--	--	--	--	--	--	----	----	---	---	---
Gel # 4	----	----	----	----	----	----	---	----	----	---	----

* Affected area around the polymer.

-- 10-15% --- 5-10% ---- <5%.

Toxicity (inhibitory) test against the most efficient micro-organisms in plants media (rizosphere).

This study was carried out on the following canditated hydrogels, taking into consideration that the concentration of applied hydrogels in petri dishes are higher than that normally used in soil conditioning .

Gel #1, was prepared under expt. Conditions (0.12 H_2O_2/RS ratio, 0.1% ferrous ammonium sulfate, 2AN /RS, for 3 h, at 50°C followed by alkali hydrolysis), using AcOH for neutralize the pH-value to ~ 6-7

Gel #2, was prepared under expt. Conditions (0.12 H_2O_2/RS ratio, 0.1% ferrous ammonium sulfate, 2AN /RS, for 3 h, at 50°C followed by alkali hydrolysis), using HNO_3 for neutralize the pH-value to ~ 6-7

Gel #3, was prepared under expt. Conditions (1.8% persulfate, 0.9% bisulfite, 2AN/RS ratio, for 3 h, at 45°C, followed by alkali hydrolysis), using HNO_3 for neutralize the pH-value to ~ 6-7.

Gel #4, was prepared by grafting the partially cyanoethylated RS, under the same conditions of hydrogel # 3, using AcOH for neutralize the pH-value to ~ 6-7.

Data presented in Table 10 are mean of three replications (four pieces/each plate=12 determinations). These data indicate that the prepared hydrogels having no-toxic effect for most of the beneficent micro-organisms in plant rhizosphere, and they can be safely used as soil conditioners. Gels no. 2 and no. 4 may be prepared to be used for most crops; while gels # 1 and # 3 can be used with crops except legumes.

Conclusion

From all the foregoing results it could be concluded that, the approach of grafting had a significant effect on water absorption affinity of the hydrogels produced. Whereas, using Ferrous sulfate-H_2O_2 initiation system rendered hydrogel with a relatively higher absorption capacity towards both distilled and Nile water than using persulfate-persulfite initiation system. Precyanoethylation of RS, especially on using AcOH for adjusting the pH of hydrogel, promoted the utilization of persulfate-persulfie initiation system (adsorption capacity increased from 2602.5 to 3788.7 for Nile water and from 2121.9 to 7061.4 for distilled water). These hydrogels have no-toxic effect for most of the beneficent micro-organisms in plant rhizosphere, and they can be safely used as soil conditioners.

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Isolation of putative LAR (leucoanthocyanidin reductase) gene fragment differentially expressed in *Jatropha curcas* L. leaves

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Abstract

Jatropha curcas Linn. (Euphorbiaceae) is a shrub, which is widely distributed especially in tropical areas of Africa. Compounds that have been isolated from *J. curcas* leaves include flavonoids, sterols their glucosides and alkaloids. Many of both structural and regulatory genes included in secondary metabolite pathways have been cloned from several model plants. Leaves at four different growing stages were collected and cDNAs obtained were analyzed by PCR applying specific primers. All the reactions were performed three times and only repeatable fragments were taken into account for sequencing. Obtained sequences were compared in EMBL databases in order to identify their homology and their putative function. A total of 45 polymorphic fragments were collected. Twenty-six of them showed homology with ESTs (Expressed Sequence Tagged), while other 19 sequences showed to be homologous to genes involved in secondary metabolism and polyphenol pathways. The results obtained underline a different kinetic expression of the gene fragment isolated. They are especially expressed at different growing stage. In particular, applying LAR gene specific primers, an amplicon of 945bp was obtained. It is 98.4% homologous to the gene previously isolated in *V. vinifera* leaves. These interesting preliminary results are the first step for study and isolate other genes involved in polyphenol pathway in *Jatropha curcas*.

Key words: *Jatropha curcas*, LAR gene, Flavonol pathway

Introduction

Jatropha curcas L., one of the 175 species in genus *Jatropha* of the family Euphorbiaceae is a perennial small tree or large shrub native to tropical America and it is distributed throughout the tropics and subtropics of Asia and Africa (Natarajan et al., 2010).

Leaves and nuts of *Jatropha* (Figure 1) are toxic (they contain phorbol esters and curcin), nuts are sometimes roasted and dangerously eaten. However, a non-toxic variety is reported to exist in Mexico and Central America where is used for human consumption after roasting, and does not contain phorbol esters (Benge, 2006).

Jatropha is known as the physic or purging nut for its use as purgative/laxative and it is widely known as medicinal for treatment of a variety of diseases. Preparations of all parts of the plant, including seeds, leaves and bark, fresh or as a decoction, are used in traditional medicine and veterinary purposes. The methanol extract of *Jatropha* leaves showed potent cardiovascular action in animals and might be a source of beta-blocker agent for humans. The latex of *Jatropha* contains alkaloids including jatrophine, jatropham and curcain with anti-cancerous properties. The oil has a strong purgative action and is widely used to treat skin diseases and to soothe pain from rheumatism. The 36% linoleic acid (C18:2) content in *jatropha* kernel oil is of possible interest for skincare (Islam et al., 2011).

Compounds isolated from J. curcas leaves include the flavonoids apigenin and its glycosides vitexin and isovitexin, the catechin, the sterols stigmasterol, β -D-sitosterol and its D-glucoside (Mousumi and Bisen, 2008). Furthermore, *J. curcas* leaves contain steroid sapogenins, alkaloids, the triterpene alcohol, 1-triacontanol and a dimer of a triterpene alcohol (Staubmann et al., 1999). Many of both structural and regulatory genes have been cloned from several model plants, including maize, Antirrhinum, Tobacco, Petunia and Arabidopsis and have been expressed in genetically modified

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model plants and micro-organisms (Bogs et al., 2005; Dixon and Steele, 1999; Forkmann and Martens, 2001). Nowadays nothing has been reported about gene expression in this specie.

Figure 1. *Jatropha curcas* L.

Aerial parts containing organic acids (*o* and *p*-coumaric acid, *p*-OH-benzoic acid, protocatechuic acid, resorsilic acid), saponins and tannins. Amyrin, sitosterol and taraxerol were isolated from stembark. Latex contains curcacycline A, a cyclic octapeptide curcain (a protease) whereas curcin, a lectin Phorbolesters Esterases (JEA) and Lipase (JEB) were isolated from seeds. Roots are characterized from sitosterol and its d-glucoside, marmesin, propacin, the curculathyranes A and B and the curcusones A–D, the diterpenoids jatrophol and jatropholone A and B, the coumarin tomentin, the coumarino-lignan jatrophin as well as taraxerol (Garg et al., 2011).

The polyphenolic compounds, known as condensed tannins or proanthocyanidins (PAs), are plant secondary metabolites synthesized via the flavonoid biosynthetic pathway. They occur in a wide range of plants and play an important role in defence against herbivores (Harborne and Grayer, 1993; Peters and Constabel, 2002). PAs can protect ruminants against pasture bloat (McMahon et al., 2000) and act as antioxidants with beneficial effects for human health including protection against free radical-mediated injury and cardiovascular disease (Bagchi et al., 2000; Lin et al., 2002; Milella et al., 2011a; Russo et al., 2012). Polyphenols were also used as marker in genomic and metabolomic comparision (Milella et al., 2011b) In red wine, PAs play an important role in wine quality, contributing to mouthfeel and colour stability (Glories, 1988). Thus, from both a nutraceutical and a food quality perspective, it is important to understand the mechanisms leading to the formation of PA polymers and how this is regulated by the plant (Bogs et al., 2005). PA biosynthesis is part of the flavonoid pathway that also produces anthocyanins and flavonols.

The objectives of this study were: isolate good quality RNA from leaves at different ripening stages; synthesize cDNA in *Jatropha curcas* L.; amplify expressed genomic sequences; identify some of them potentially involved in polyphenol biosynthetic pathways.

Materials and Methods

Plant Material

Leaves at four different growing stages were collected: young (Y), unripe (U), fully grown (F) and old (O). Leaves were immediately frozen in liquid nitrogen and stored at -80°C.

RNA Extraction and cDNA Synthesis

Total RNA was extracted starting from 0.5 g of leaf tissue with the following extraction buffer: 0.2 M Tris-HCl pH 8.5; 0.35 M NaCl; 7 M Urea; 0.2 M EDTA pH 8; 2% (w/v) SDS. To obtain highly purified RNA, during the cleaning steps, particular attention was placed. The synthesis of the cDNA was carried out following the protocol described in Ovesna et al. (2012).

Primer design and amplification

cDNAs obtained were analyzed by PCR applying specific primers related to polyphenol pathway (Table 1). They were obtained aligning different nucleic acid sequences of the same gene previously isolated in other species. Clustal V software was used to determinate the highest conserved gene regions and to design specific oligonucleotides (Higgins et al., 1992). PCR reactions were carried out in 50 µl final volume using 0.3 units of Taq polymerase, 10 mM Tris-HCl pH 8.1, 50 mM KCl, 250 mM dNTPs, 3 mM $MgCl_2$ and 2 mM primers, 0.5 ng of cDNA for 45 cycles with the following thermal profile: 94°C for 1.10 min, 44°C for 1.00 min, and 72°C for 2.00 min. Final elongation step was done at 72°C for 5.00 min. Amplified fragments were fractioned through 1.6% agarose gel containing 0.5 mg/ml of ethidium bromide. All the reactions were performed three times and only repeatable fragments were analyzed for sequencing.

Table 1. Some of the primers used for PCR amplification.

Primer	Sequence	N°bases	CG %
LAR for	5'-CATGGACAACACTCGATTAGCCTAC-3'	25	48
LAR rev	5'-TGTTGATGACAAAAGTAATGGGG-3'	23	40
CHI for	5'-ACACCTGCTGGAGTTGCCCA-3'	20	60
CHI rev	5'-AAAGAATGGGGCCCAGCCCA-3'	20	60
LDOX for	5'-CATTGGAGGAAGATGAGAGAATCATGAC-3'	28	42,8
LDOX rev	5'-GTCACAATCTGGCGCCAATCCT-3'	22	54,5

Sequencing and alignment of cDNA isolated fragments

Differentially expressed amplicons obtained were excised from the gel and eluted using "Quantum prep freeze 'N sequence DNA gel extraction spin columns" Bio-rad kit. The recovered fragments were cloned using pGEM-T Easy Vector Systems (Promega). Cloned cDNAs were sequenced using Sequenase kit applying Sp6 primer.

Sequences were analyzed using BLASTN algorithm and compared with EMBL databases in order to identify their homology and putative function with those previously isolated.

Results and Discussion

The protocol applied for RNA extraction and cDNA synthesis demonstrated to be very effective in order to isolate good quality RNA and to generate cDNA starting from sampled tissues in *Jatropha curcas*.

Applying specific oligos, related to the polyphenol pathway, clear polymorphisms were observed. This result underlines a different kinetic of investigated gene expression. A total of 45 polymorphic fragments were collected, they included only the reproducible amplification products that showed polymorphisms among leaf growing stages. In order to confirm the size and the uniqueness of the isolated fragments (Figure 2), they were re-amplified. Twenty-six of them showed homology with ESTs (Expressed Sequence Tagged), while other 19 sequences showed to be homologous to expressed products involved in secondary metabolism and polyphenol pathway (Table 2).

The results reported in Table 2 shown the homologies found with genes involved in the flavonoid pathway. In particular, the fragments isolated from fully grown (F) stage shown homologies with different compounds involved in the synthesis of PAs and anthocyanins: leucoanthocyanidin reductase (LAR), Leuco Anthocyanidin Dioxygenase (LDOX) and Flavone synthase (FLS). Previous studies were focused on LAR gene, which plays a key role in catechin biosynthesis (Bogs et al., 2005). Using specific primers an amplicon of 945bp was obtained. It is 98.4% homologous to the gene previously isolated in *V. vinifera* leaves 1086bp (Bogs et al., 2005).

Table 2. Isolated fragment homologies.

Code	Protein	Growing stage	Access.
6C1-W2	Leuco Anthocyanidin Reductase (LAR)	F	L81312
5CY-Y2	Chalcone Isomerase (CHI)	U, F	Q949N3
20-Y2	Leuco Anthocyanidin Dioxygenase (LDOX)	F	Q9ST16
19-R2	Flavone synthase (FLS)	U, F, O	Q6R4C2

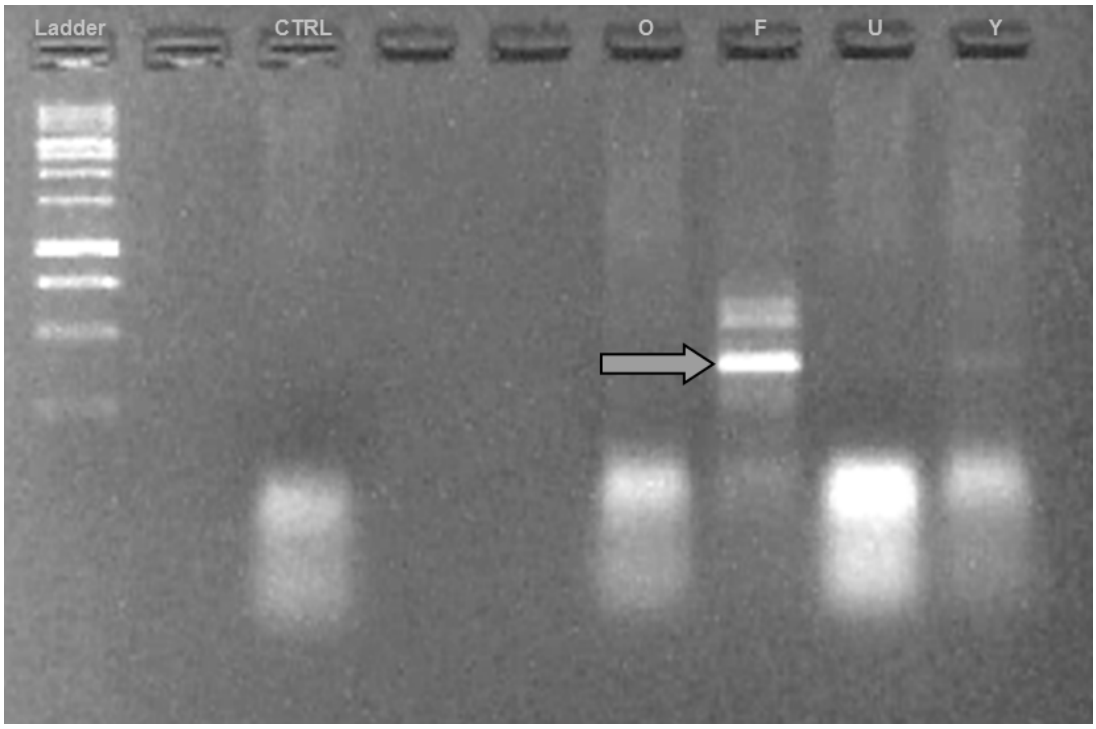

Figure 2. LAR amplification product (indicated by arrow) obtained by PRC reaction.

In this study, we show that the cDNA coding for LAR gene is expressed during PA synthesis in *J. curcas* and are regulated in a temporal manner and it is genotype dependent. Synthesis of PA polymers is believed to occur by addition of an intermediate derived from a flavan-3,4-diol (such as leucocyanidin) to a flavan-3-ol terminal unit (such as catechin or epicatechin) with sequential addition of further extension subunits as the polymer lengthens. Taking into account the stage of the leaves analyzed it is possible to see that the LAR expression is present only in the ″F″ stage. Unlike, the FLS gene it is expressed in all stages evaluated except than Y stage (young leaf).

These interesting preliminary results are the first step for study and isolate other genes involved in polyphenol pathway in *J. curcas*.

cDNAs will be insert in a plasmid and expressed *in vitro* to confirm protein structure and biological function.

Is particularly important to isolate and characterize genes regulating the biosynthesis of secondary metabolites of interest, such as the isolated fragment showing homology with the sequence of the LAR enzyme.

Whereas the flavan-3-ol biosynthesis had traditionally been considered to be the product of leucoanthocyanidin reductase (LAR), converting leucocyanidin to catechin with an epimerase then converting catechin to epicatechin (Stafford, 1990) and since LAR activity has been reported in several plants and its activity correlated with PA accumulation (Stafford, 1990; Joseph et al., 1998; Marles et al., 2003), could be interesting to aimed further studies to investigate the level of expression of several genes involved in flavonoids patway in different stages of growth and in particular assess expression of the LAR gene in fully grown (F) stage.

Conclusion

The flavonoid biosynthetic pathway is one of the most important metabolic systems in plants. The development of genomics datasets for organisms that include Arabidopsis, the moss *Physcomitrella patens* (http://www.moss.leeds.ac.uk/), and the model legume *Medicago truncatula* (http://www.noble.org/medicago/), are also offering opportunities to examine this metabolic model system from entirely new perspectives. As with any good model, each new piece of information appears to raise a number of unanticipated and intriguing questions. At the same time, new tools are providing the opportunity to consider flavonoid biosynthesis, not as an assemblage of independent components, but as part of a large, complex, and tightly orchestrated metabolic network. The ability to now consider flavonoid enzymes in *Jatropha curcas*, for the very first time, and to examine the

interdependence of the pathways of secondary metabolism using genomic, proteomic, and metabolic profiling methods are likely to move us much more rapidly toward this end. Moreover, others studies of these aspects and detailed knowledge of the mechanisms regulating these physiological processes may be a key to understand and then manage those processes for obtaining products that can be used in areas such as nutraceutical and/or producing compounds of vegetable active against some diseases. It is quite clear that, even for much-studied "old" pathways like flavonoid biosynthesis, these are exciting times.

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ANIMAL SCIENCE

Phenotypic variability of two principal Algerian camel's populations (Targui and Sahraoui)

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Abstract

The camel population and its phenotype variability are not well described in Algeria. The studies at the colonial period were based primarily on a nomenclature associated with the names of tribes rather than the measurement of phenotypic parameters. The present study aimed to define the discriminating parameters of the two main populations described in Algeria, namely the Sahraoui and the Targui breed. An initial analysis was achieved for each population (95 Targui and 95 Sahraoui) to demonstrate an internal variability in each group that includes two to three subpopulations. A second analysis was focused on two populations combined. From the measurements of 190 adult animals, attached to one or the other population, a canonical discriminant analysis was applied to determine the most discriminant parameters, (abdominal circumference, chest circumference, height at withers and turn spiral) and to evaluate the percentage of classified assets. These four parameters were sufficient to distinguish the two populations with 98.5% camels well classified. For each of the populations and subpopulations, standard body measurements are proposed.

Key words: Algeria, Body measurements, Discriminant analysis, Dromedary, Phenotype

Introduction

In Algeria, the Sahara covers more than 85% of the total area. The dromedary is the only species capable to valorize this desert ecosystem (Chehma et al., 2008). The total number of the camels is estimated by the Ministry of Agriculture in 2010 to more than 300000 heads. Algerian camel populations are poorly described and the only indications were based on studies performed at the colonial period (Cauvet, 1925; Boué, 1946; 1948). In fact, the nomenclature of these populations was more related to the names of tribes who breed them (Chambi, Targui, Reguibi) than a distinction based on phenotypic characteristics.

Across the world, there were some reports done on the phenotypic diversity of camel populations, like those of Ishag et al. (2011) in Sudan, Faye et al. (2011) and Abdallah and Faye (2012) in Saudi Arabia.

Moreover, the two main tribes known for camel breeding in Algeria were the Chaamba in the northern Sahara and the Touareg in the central Sahara, breedingcamels type Sahraoui and Targui respectively (Ben Aissa, 1988; Oulad Belkhir 2008).

Thus, our study aimed to identify the principal phenotypic characteristics of these two camel populations (Sahraoui and Targui) the most dominant in Algeria in order to report the eventual variability between and within populations.

Materials and Methods

Study area

Our field investigations mainly involved two large Saharan regions known for the camel breeding in Algeria, which are: the Ouargla region (North-Western Sahara) and the region of Tamanrasset (Central Sahara).

Animals

The study of the two populations, Sahraoui and Targui, was focused on adult animals. The appreciation of their age was made on the basis of dentition. Therefore, 95 individus (males and females) were studied for each population and each region.

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Measurements

On each animal, twelve (12) measurements were performed according to standard techniques (Marmet, 1983; Pagot, 1985; Delaine Pagot, 1959), via a meter-ribbon, with the exception of the height at withers which was performed with toise. The different measurements performed on the animals were abdominal circumference (AC), heart girth (HG), the height at withers (HW), turn spiral (TS), the length of the head (LH), length of the neck (LN), the height at the hump (HH) and the length of the hind limbs (LHL).

Statistical analyses

Statistical analyzes included two stages, (i) an analysis of internal variability in each population (Sahraoui and Targui) and (ii) a comparative analysis to determine the variability between populations.

The analysis of internal variability included:

The description of the mean and standard deviation for each of the body measurements,

- The correlations between different body measurements for each population (Pearson correlation),

- Automatic classification of each population to identify homogeneous sub-populations (hierarchial ascending classification),

The analysis of the variability between populations consisted of:

Identifying the differences between body measurements (ANOVA).

Identifying the most discriminating body measurements for both populations (method of stepwise discriminant analysis) and determine the percentage of well classified according the parameters retained by the discriminating model

Performing an automatic classification of all two populations (k-means method).

Establishing a contingency table between population (Targui or Sahraoui) and classes from the previous step and test the independence between population and classes by the Chi² test.

These analyzes were performed using the software XLStat (Addinsoft ©).

Results and Discussion

Differences in the body measurements between camel populations

Wither height, abdominal circumference length of the head and neck were significantly greater in the Targui compared to Sahraoui (Table 1).

Table 1. Mean ± standard deviation of body measurements of camel populations Sahraoui and Targui (in cm).

	Sahraoui	Targui
HW	1,781 ± 0,119 [a]	1,922 ± 0,187 [b]
HG	1,901 ± 0,248 [a]	1,815 ± 0,223 [a]
AC	1,638 ± 0,199 [a]	2,200 ± 0,258 [b]
HH	2,386 ± 0,271 [a]	2,140 ± 0,200 [a]
TS	2,285 ± 0,238 [a]	2,286 ± 0,302 [a]
LHL	1,745 ± 0,165 [a]	1,977 ± 0,265 [a]
LN	1,028 ± 0,106 [a]	1,092 ± 0,126 [b]
LH	0,500 ± 0,047 [a]	0,521 ± 0,051 [b]

a, b: the difference of letters on a line attests to a significant difference at P <0.05

Table 2. Correlation matrix between the measurements in each population camel. The characters in bold are significant at P <0.05.

Sahraoui	HG	CT	CA	HB	TS	LMP	LC	LT
HG	**1**	**0,333**	**0,433**	**0,267**	**0,597**	**0,717**	**0,394**	**0,483**
CT	**0,333**	**1**	**0,599**	**0,672**	**0,517**	-0,056	**0,457**	-0,003
CA	**0,433**	**0,599**	**1**	**0,676**	**0,487**	0,202	**0,512**	0,113
HB	**0,267**	**0,672**	**0,676**	**1**	**0,583**	-0,024	**0,375**	0,016
TS	**0,597**	**0,517**	**0,487**	**0,583**	**1**	**0,451**	**0,344**	**0,311**
LMP	**0,717**	-0,056	0,202	-0,024	**0,451**	**1**	0,176	**0,519**
LC	**0,394**	**0,457**	**0,512**	**0,375**	**0,344**	0,176	**1**	**0,293**
LT	**0,483**	-0,003	0,113	0,016	**0,311**	**0,519**	**0,293**	**1**
Targui	HG	CT	CA	HB	TS	LMP	LC	LT
HG	**1**	**0,736**	**0,642**	**0,792**	**0,584**	**0,332**	**0,571**	**0,623**
CT	**0,736**	**1**	**0,812**	**0,737**	**0,698**	**0,404**	**0,653**	**0,693**
CA	**0,792**	**0,737**	**1**	**0,782**	**0,720**	**0,452**	**0,632**	**0,664**
HB	**0,642**	**0,812**	**0,782**	**1**	**0,645**	**0,315**	**0,600**	**0,612**
TS	**0,332**	**0,404**	**0,315**	**0,452**	**1**	**0,596**	**0,539**	**0,497**
LMP	**0,584**	**0,698**	**0,645**	**0,720**	**0,596**	**1**	**0,692**	**0,658**
LC	**0,623**	**0,693**	**0,612**	**0,632**	**0,692**	**0,539**	**1**	**0,683**
LT	**0,571**	**0,653**	**0,600**	**0,664**	**0,658**	**0,497**	**0,683**	**1**

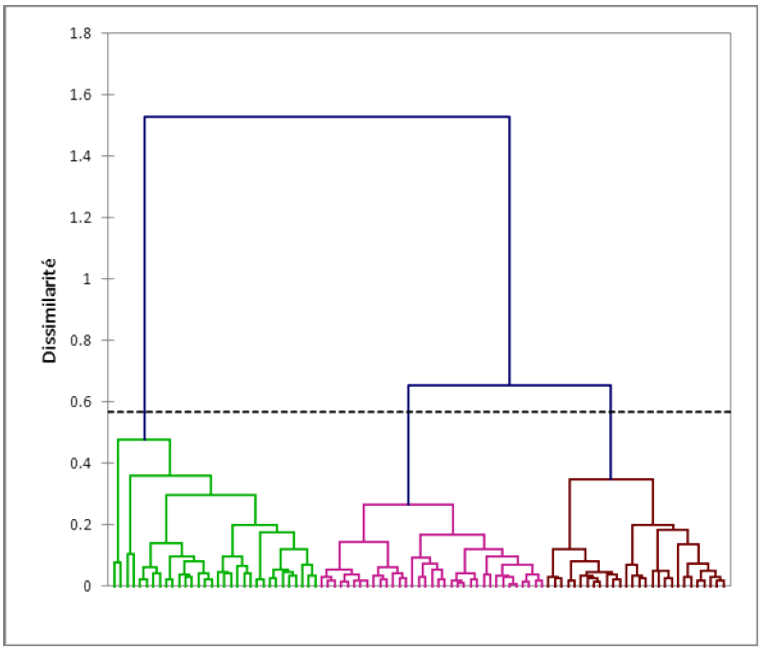

Figure 1. Hierarchical clustering of Saharaoui's dromedary based on their measurements.

Table 3. Mean body measurements of different classes of the Sahraoui population.

classe	HW	HG	AC	TS	LN	LH
A	1,799 [a]	1,675 [a]	1,480 [a]	2,267 [a]	0,973 [a]	0,522 [a]
B	1,770 [a]	1,980 [b]	1,730 [b]	2,169 [a]	1,060 [b]	0,495 [b]
C	1,773 [a]	2,060 [b]	1,704 [b]	2,453 [b]	1,052 [b]	0,480 [b]

a, b: the difference of letters on a line attests to a significant difference at P <0.05

Correlations between the measurements

The correlations were generally positively significant between different measurements of the animal in the two populations, but more significantly in the Targui population which had, therefore, a better proportionality in its measurements (Table 2). The height at the withers and the spiral turn measurements appeared more correlated to the other measurements.

Classification of the Sahraoui population

The hierarchical classification of the body measurements performed on the Sahraoui population has identified a clear partition in 3 different classes expressing 71.4% of the variance (Figure 1).

The three Sahraoui subpopulations could be described as follows (Table 3):

- A group of 32 animals rather narrow but with a lower thoracic and abdominal circumference and a longer neck
- A group of 35 animals rather large but with a higher thoracic and abdominal circumference and a shorter neck
- A group of 28 dromedaries to raise spiral turn, a large chest circumference but with a small head.

Classification of the Targui population

The hierarchial classification of body measurements performed on the Touareg population identified a partition in 3 classes also clearly distinct and expressing 64.9% of the variance (Figure 2).

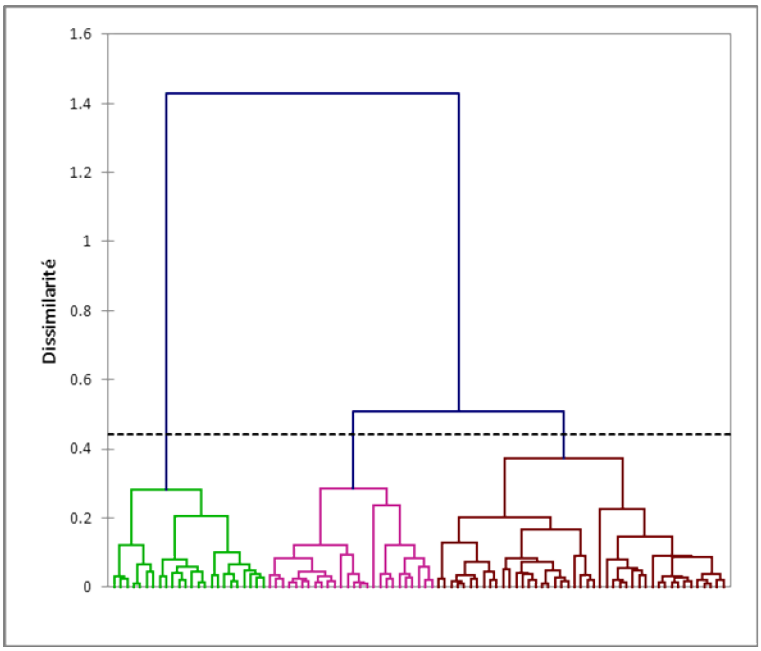

Figure 2. Hierarchical clustering of Targui's dromedary on the basis of their measurements

Table 4. Mean measurement of the different classes of the population Targui.

Classe	HW	HG	AC	TS	LN	LH
A	1,965[a]	1,980[a]	2,374[a]	2,526[a]	1,103[a]	0,542[a]
B	1,990[a]	1,840[b]	2,189[b]	2,302[b]	1,123[a]	0,545[a]
C	1,980[a]	1,916[b]	2,346[a]	1,996[c]	1,020[b]	0,519[b]

a,b,c the difference of letters on a line attests to a significant difference at P <0.05

The analysis of variance applied to the three sub-populations indicates that they are distinguished as follows:

- A population of 26 camels of large size (HG, AC, TS, LN, LH large size),
- A population consisting of 45 animals with a long neck and long head but thoracic and abdominal circumferences less developed.
- A group of 24 dromedaries characterized by low turn spiral, a small head and neck of modest size. The thoracic and abdominal circumferences appeared intermediate.

Parameters discriminating the 2 populations

The stepwise discriminant analysis identified the measures in order of importance in their discriminating power. In order, the most discriminant variables were: the abdomen circumference, the circumference of the chest, shoulder height and turn spiral. The addition of other measurements didn't improve the discriminating power. These four parameters were sufficient to distinguish the two populations with 98.5% of well-classified animals. The percentage of well-classified was identical in the two populations.

Confusion matrix

In the last step, it was conducted a global analysis of the whole camel population (190 dromedaries) by the method of k-means which classified individuals according to a random number of classes. The result gave five morphological types (classes of measurements) which were faced to the two populations Targui and Sahraoui. This global analysis confirmed the strong phenotypic dichotomy since the classes 1 and 2 respectively comprised 100 and 98.7% of Sahraoui camels population, and Class 3, 4 and 5 respectively congregating 95.6, 96.1 and 100% of Targui camel population (Figure 3).

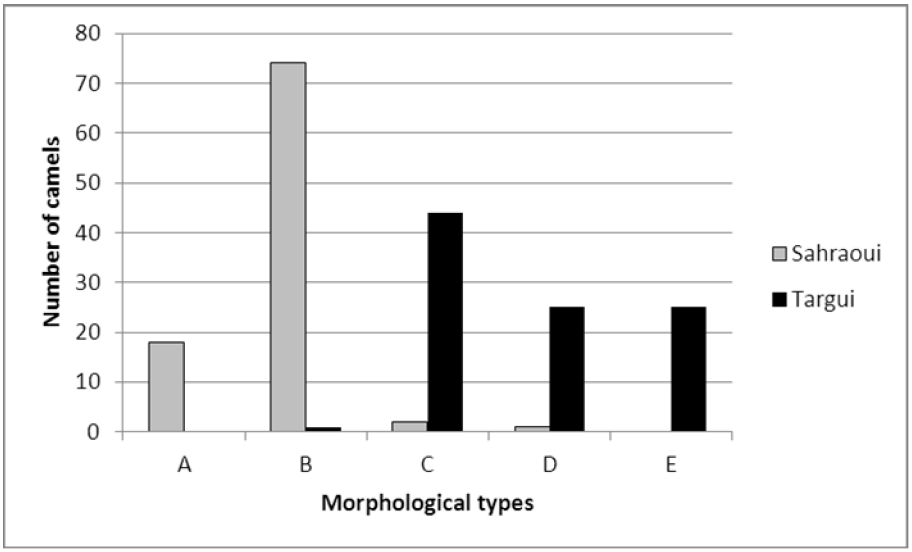

Figure 3. Distribution of morphological types among the camel populations Targui and Sahraoui.

These morphological types showed the following characteristics (Table 5):

- Type A: small morphology for all measurements
- Type B: the larger, medium-sized animals, with high thoracic perimeter, and low abdominal circumference and small head,
- Type C: large animal morphology for all dimensions,
- Type D: average size of animals with long neck and well-developed head,
- Type E: larger animals differing from the previous type by its thoracic and abdominal perimeters stronger but a spiral turn smaller

It appears from these results a clear phenotypic difference between Sahraoui and Targui populations. The first appearing smaller, skinny but relatively long (the spiral turn appears relatively long in some individuals) with a smaller head and a neck less developed (photo 1), the opposite of the Targui with larger dimensions (photo 2).

Discussion

The coat color parameter, whitish in the Targui population, brown to dark brown in the Sahraoui, is not the only discriminating character. Our results based on measurements only, showed morphologies very well-marked to classify over 98% of animals solely on the criteria of thoracic perimeter, abdominal perimeter, spiral turn and height at the withers. However, our results also showed that through phenotypic body measurements, the two studied populations (Sahraoui and Targui) were not homogeneous and morphological subpopulations for each of them were existing and were relatively distinct. This can be explained by the fact that these two populations have been named on the basis of their tribal affiliation and not on phenotypic parameters.

Table 5. Mean measurements in the different classes of the Algerian camel population (m).

	nb	HW	HG	AC	TS	LN	LH
typeA	18	1,670^{d}	1,543^{d}	1,367^{d}	2,010^{c}	0,906^{d}	0,493^{c}
typeB	75	1,801^{c}	1,992^{a}	1,691^{c}	2,325^{b}	1,057^{b,c}	0,499^{c}
typeC	46	2,031^{a}	1,981^{a}	2,367^{a}	2,511^{a}	1,132^{a}	0,548^{a}
typeD	26	1,911^{b}	1,761^{c}	2,080^{b}	2,265^{b}	1,086^{b}	0,539^{a,b}
typeE	25	1,964^{b}	1,871^{b}	2,313^{a}	1,976^{c}	1,022^{c}	0,518^{b}

a,b,c,d : the difference of letters on a line attests to a significant difference at P <0.05

Photo 1. Dromedary of population Sahraoui.

Photo 2. Dromedary of population Targui.

These subpopulations may also be associated to a set of dietary practices, themselves related to the quality of rangeland whose influence on morphological development of animals was widely noted. Based on discriminant analysis of two populations, it appeared that the discriminating variables were especially those related to height (height at the withers) and the circumference of the body (abdominal circumference, chest circumference, and spiral turn). In fact, the Targui population is recognized as an animal higher and used especially as racing animal (especially animals with high thoracic perimeter and low abdominal perimeter) and riding, contrary to Sahraoui which is an animal rather more robust and heavier, used for packing and meat production (Ben Aissa, 1988; Oulad Belkhir, 2008).

However, this differentiation is therefore based on usage rather than on a reasoned selection scheme piloted by farmers. The relationship between morphology and a set of performances (production, growth, reproduction) obviously deserves to be deepened. Current studies based on tools of molecular genetics, should confirm or refute these results in order to clarify whether the observed phenotypic differences were based on genetically distinct populations, which, according to the preliminary results did not appear to be different (Burger, personal communication).

Conclusion

This work is a small contribution to the knowledge of the phenotypic variability of the two principal camel populations in Algeria. The results reported the existence of subpopulations even within each population. And the discriminant analysis direct us to the parameters related to the uses of these two populations. However, for further refining this study, it is necessary to consider the performance characteristics (meat production, milk productivity and numerical productivity), with the perspective to lead to a kind of standards Algerian camel races.

Acknowledgment

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ANIMAL SCIENCE

Software for planning loose yards and designing concrete constructions for dairy farms in arid and semi-arid zones

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Abstract

To plan and design dairy housing, several calculations should be made; this requires time and efforts, with the possibility of making mistakes. The objectives of this paper are to develop a tool to assist the designers in planning and designing dairy housing in arid and semi-arid zones, to save time and efforts, and to provide a new design model. Two mathematical models were developed to plan and design corral systems and the required concrete constructions. Subsequently, an electronic spark map (decision tree) was developed for each mathematical model, and then the mathematical models were integrated into the electronic spark maps. Afterwards, C# (C Sharp) programming language was used to develop the software by integrating the electronic spark maps, and making the user interface. The developed software is able to plan and design the housing system (corrals system), specify corrals and house dimensions, and compute the required amounts of construction materials (iron rods, cement, sand, and gravels) to build the required concrete base. Furthermore, it calculates the capital investment and the fixed, variable, and total costs of the construction. Data of 6 dairy farms were used to validate the model, and to evaluate the software. The differences between actual and calculated values were determined, and the standard deviations were calculated. The coefficients of variation (COVs) range between 3% and 7%.

Key words: Arid zones, Dairy farms, Loose yards, Mathematical modeling, Precision livestock farming

Introduction

An open housing system, in hot climates, consists of a yard shaded by a roof. This system allows air to move in the space between the roof and the floor performing natural ventilation which enhances dairy cows' microclimate especially by increasing the cowshed height to 8 m (Hatem et al., 2006). Walthes and Charles (1994) stated that yards or corral systems are used in hot climates and they allow 46 to 56 m^2 per cow; on the other hand, Hatem et al. (2004a,b) and Samer (2004) stated that the standard value is 20 to 25 m^2 per cow. Samer (2010a) investigated and compared three dairy corral designs with 25 m^2 per cow for each design. Samer et al. (2007) developed a dairy farm foundation model. Ahachad et al. (2008) mentioned the most influential parameters on heat stress, which are: ventilation, shape, orientation, number of occupants etc. An important key issue is the area allotted per animal which when increased -up to some limits- the stress decreases. Bartali (1999) stated that reinforced concrete is obtained by adequately mixing in specific proportions aggregates (gravel and sand), cement, and water. Lindley and Whitaker (1996) elucidated that water : cement ratio is 0.53 l/kg and cement : sand : gravel mass ratio is 1:2.2:3.7 for floors, driveways, structural beams, and columns.

In order to accelerate analyses and improve on-farm decision-making, it is necessary to develop computer tools that have the ability to preprocess the data so as to produce value-added information (Lacroix et al., 1998). The common form of an expert system is a computer program, with a set of rules or equations that analyzes information or data supplied by the user, about a specific problem, and recommends one or more courses of user action.

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The expert system may also provide mathematical analysis of the problem (Giarratano and Riley, 2005).

The objective of this study is to develop software to plan and design corral systems and the required concrete constructions, to compute the required amounts of construction materials, and to calculate the costs.

Materials and Methods

The tool is developed by integrating two mathematical models into two electronic spark maps. The mathematical models were developed using the plans, designs, parameters, variables, and constant values of corral systems and the concrete structures available in the references. Subsequently, MS-Excel is used to develop the electronic spark maps of the mathematical models, and to show the results of the input settings automatically.

Afterwards, C# language (C#, 2005), which is an object-oriented programming language, was used to develop the software by integrating the electronic spark maps of the mathematical models to form the back diagram code of the software, and then to develop the user interface. This methodology represents a new approach for developing programs using decision trees and mathematical models for practical implementation.

The software was validated and evaluated using data of 6 Egyptian dairy farms, as examples of dairy farms in arid and semi-arid zones. The relative differences between actual values and calculated values were determined for corral and house dimensions, concrete base dimensions, concrete volume, gravels volume, cement mass, sand volume, and iron rods mass, and then the averages of relative differences were computed. Subsequently, the standard deviations (σ) and the coefficients of variation (COV) were determined.

Design model

The objective of making a design model (DM) is to assist the designers in designing the corrals. The following mathematical model was developed to be the core of the software (for nomenclature see Tables 1 through 4):

Corral dimensions

$$W_C = N_{CC} \times (\frac{1}{N_{CFP}}) \times L_{FB} \quad (1)$$

$$A_C = N_{CC} \times A_{AC} \quad (2)$$

$$L_C = \frac{A_C}{W_C} \quad (3)$$

$$N_{HC} = \frac{N_{CH}}{N_{CC}} \quad (4)$$

where, $4 \le W_C \le 15$ (5)

$$10 \le N_{CC} \le 15 \quad (6)$$

$$1 \le N_{CFP} \le 3 \quad (7)$$

$$0.75 \le L_{FB} \le 0.95 \quad (8)$$

$$20 \le A_{AC} \le 25 \quad (9)$$

The available corral systems are: two sides of corrals, one side of corrals, and one corral where the suitable roof structure for each corral system was presented by Samer et al. (2008, 2008a, 2012). The shade structure should provide 85% shading, i.e. the roof should shade 85% of the corral/yard area (Samer, 2010b,c). If planning several cowsheds in the same farm, it is recommended to consider the farm planning method developed by Samer et al. (2008b), where it is preferred that the sheds have the same orientation (east-west in hot climates) with at least 30 m between any two adjacent sheds. Each corral system has its own mathematical model, but the same general information should be considered for the three systems:

$$0.15 \le W_{RC} \le 0.20 \quad (10)$$

$$0.15 \le W_{BB} \le 0.20 \quad (11)$$

$$W_{LB} = F(\text{feeding system}) \quad (12)$$

$$W_{FP} = F(\text{breed, manure handling system}) \quad (13)$$

The feeding places are parts of the corral. Thus, (W_{FP}) is part of (L_C).

Two sides of corrals

This corral system is suitable for large group size (Figure 1), and it has one concrete base (Figure 2), thus:

$$W_{CB} = W_{LB} + (2 \times W_{FP}) + (2 \times W_{BB}) + (2 \times W_{RC}) \quad (14)$$

$$L_H = \frac{N_{HC} \times W_C}{2} \quad (15)$$

$$W_H = (2 \times L_C) + W_{LB} \quad (16)$$

$$A_H = L_H \times W_H \quad (17)$$

if $L_C > 10$, then $H_C = 8$ (18)

if $5 \le L_C \le 10$, then $H_C = 5$ (19)

if $L_C < 5$, then $H_C = 3.5$ (20)

where, N_{HC} is an even positive number.

One side of corrals

This corral system is suitable for medium group size (Figure 3), and it has one concrete base (Figure 4), thus:

$$W_{CB} = W_{LB} + W_{FP} + W_{BB} + W_{RC} \quad (21)$$

$$L_H = N_{HC} \times W_C \quad (22)$$

$$W_H = L_C + W_{LB} \quad (23)$$

$$A_H = L_H \times W_H \quad (24)$$

if $L_C > 20$, then $H_C = 8$ (25)

if $10 \le L_C \le 20$, then $H_C = 5$ (26)

if $L_C < 10$, then $H_C = 3.5$ (27)

where, N_{HC} is a natural number

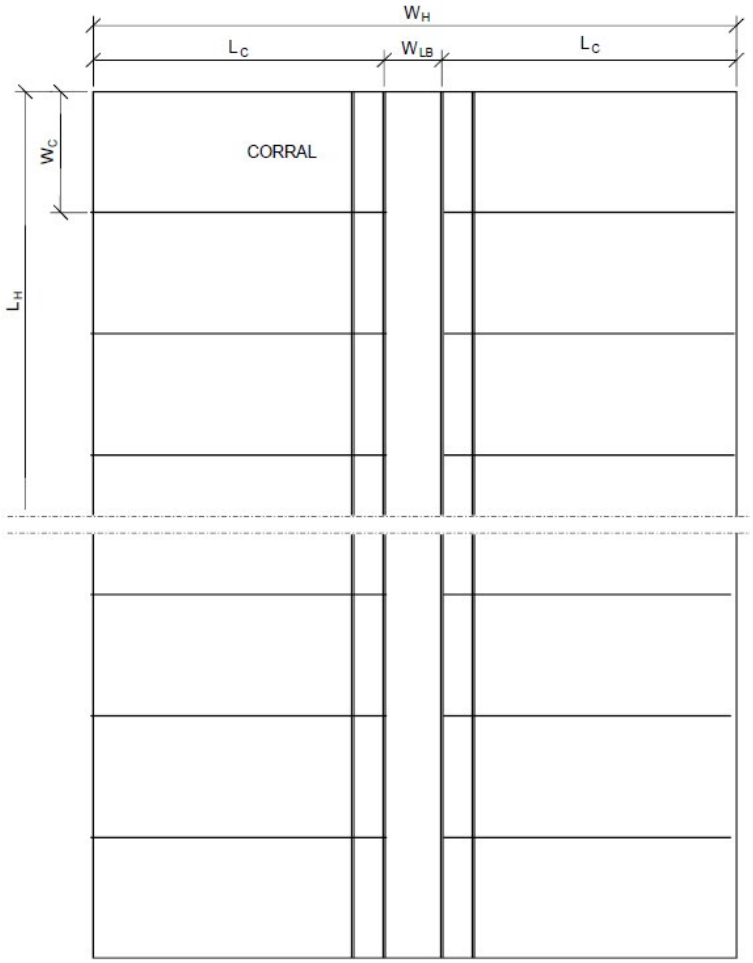

Figure 1. Two sides of corrals.

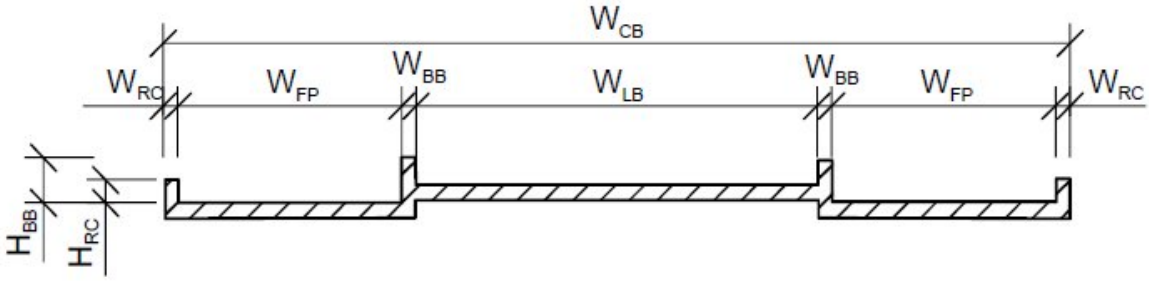

Figure 2. Concrete base for two sides of corrals.

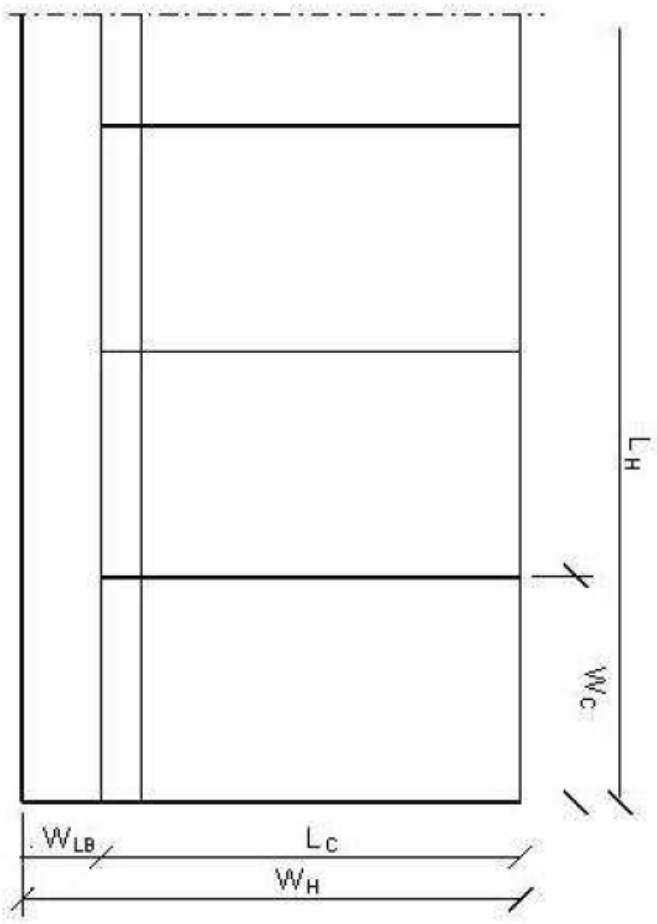

Figure 3. One side of corrals.

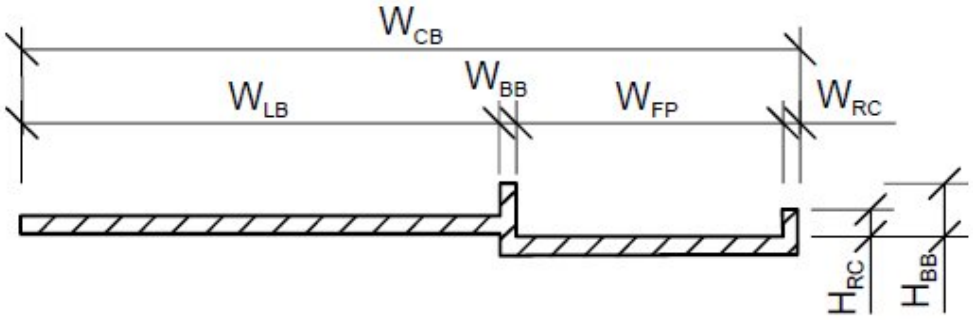

Figure 4. Concrete base for one side of corrals.

One corral

This corral system is suitable for a small group of 10-15 cows (Figure 5), and it has one concrete base (Figure 6), thus:

$$W_{CB} = W_{LB} + W_{FP} + W_{BB} + W_{RC} \quad (28)$$

$$L_H = (N_{HC} \times L_C) + W_{LB} \quad (29)$$

$$W_H = W_C \quad (30)$$

$$A_H = L_H \times W_H \quad (31)$$

if $10 \le W_C$, then $H_C = 5$ (32)

if $W_C < 10$, then $H_C = 3.5$ (33)

where,

$N_{HC} = 1$ (34)

$$N_{CH} = N_{CC} \quad (35)$$

$$10 \le N_{CC} \le 15 \quad (36)$$

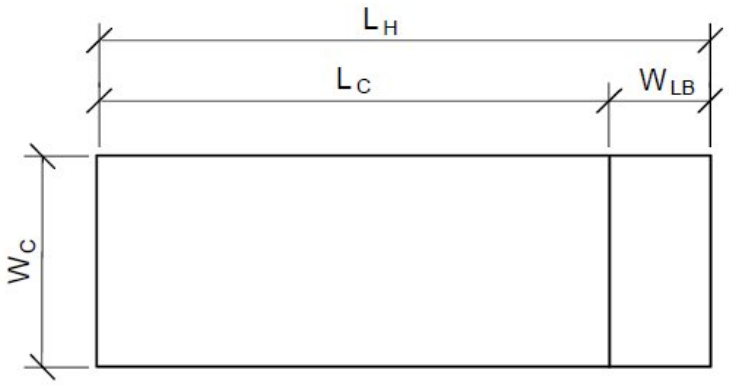

Figure 5. One corral.

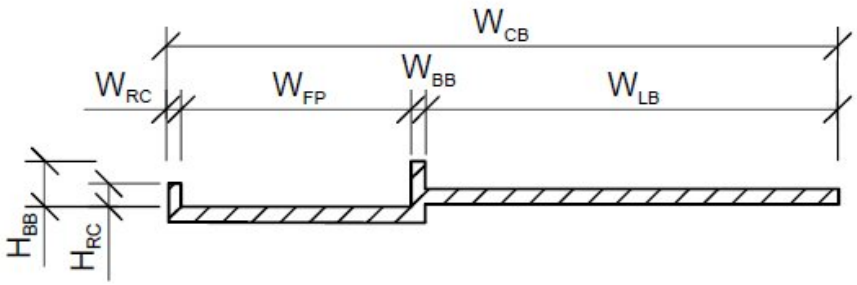

Figure 6. Concrete base for one corral.

Decision making

The designer should gather the climatic data of the location where the dairy farm will be established, such as: precipitation (mm/year), wind speed (m/s), wind direction, mean maximum temperature (°C), relative humidity (%), and sunshine (%). Then, he should make a decision to select one of the following options:

Roof materials

- If Wind Speed < 1.8 m/s and Precipitation < 80 mm/year, then:

Reed Mats, or Straw Mats

- If Wind Speed > 1.8 m/s and Precipitation > 80 mm/year, then:

Polished Aluminum (Reflection 90 - 70%)
or Isolated Aluminum, Burnt-Clay Bricks,
or Concrete (expensive), or Wood (available?)

Construction materials

- Steel Construction (recommended)
- Concrete Construction (expensive)
- Wood Construction (available?)

Orientation

- East-West (recommended in hot climates)
- North-South

Floor materials

- Feeding Place: Concrete
- Laying Place: Sand, or Chopped Straw

Shade calculations

$$A_{FP} = L_{FP} \times W_{FP} \quad (37)$$

$$R_A = \frac{A_{FP}}{A_C} \quad (38)$$

$$A_{SHF} = A_H \times R_A \times \left(\frac{S_F}{100}\right) \quad (39)$$

$$A_{SHL} = A_H \times (1 - R_A) \times \left(\frac{S_L}{100}\right) \quad (40)$$

$$A_{SH} = A_{SHF} + A_{SHL} \quad (41)$$

$$W_{SH} = \frac{A_{SH}}{L_{SH}} \quad (42)$$

where, $L_{SH} = L_H$ (43)

W_{FP} = F(breed, manure handling system) (44)

Facilities

$$N_{HF} = \frac{N_{CF}}{N_{CH}} \quad (45)$$

$$A_{tF} = (N_{HF} \times A_H) + A_M + A_{MC} + A_{FS} + A_O \quad (46)$$

Concrete base model

The objective of making such a model is to determine the values of the concrete base dimensions which lead to calculate the concrete base volume, and then the required amount of cement, iron rods, sand, and gravels. The model is also able to calculate the capital investment and the fixed, variable, and total costs.

Concrete base volume

The dimensions of the concrete base have been calculated by DM, but still the thickness and the volume:

Two sides of corrals

$$L_{CB} = L_H \quad (47)$$

$$V_{LB} = L_{CB} \times W_{LB} \times T_{LB} \quad (48)$$

$$V_{BB} = 2 \times L_{CB} \times W_{BB} \times H_{BB} \quad (49)$$

$$V_{FP} = 2 \times L_{CB} \times (W_{FP} + W_{BB} + W_{RC}) \times T_{FP} \quad (50)$$

$$V_{RC} = 2 \times L_{CB} \times W_{RC} \times H_{RC} \quad (51)$$

$$V_{CB} = V_{LB} + V_{BB} + V_{FP} + V_{RC} \quad (52)$$

where, $T_{LB} = F(\text{feeding system})$ (53)

$$T_{FP} = F(\text{breed}) \quad (54)$$

$$H_{BB} = 0.50 \quad (55)$$

$$0.20 \le H_{RC} \le 0.25 \quad (56)$$

One side of corrals

$$L_{CB} = L_H \quad (57)$$

$$V_{LB} = L_{CB} \times W_{LB} \times T_{LB} \quad (58)$$

$$V_{BB} = L_{CB} \times W_{BB} \times H_{BB} \quad (59)$$

$$V_{FP} = L_{CB} \times (W_{FP} + W_{BB} + W_{RC}) \times T_{FP} \quad (60)$$

$$V_{RC} = L_{CB} \times W_{RC} \times H_{RC} \quad (61)$$

$$V_{CB} = V_{LB} + V_{BB} + V_{FP} + V_{RC} \quad (62)$$

One corral

$$L_{CB} = W_H \quad (63)$$

$$V_{LB} = L_{CB} \times W_{LB} \times T_{LB} \quad (64)$$

$$V_{BB} = L_{CB} \times W_{BB} \times H_{BB} \quad (65)$$

$$V_{FP} = L_{CB} \times (W_{FP} + W_{BB} + W_{RC}) \times T_{FP} \quad (66)$$

$$V_{RC} = L_{CB} \times W_{RC} \times H_{RC} \quad (67)$$

$$V_{CB} = V_{LB} + V_{BB} + V_{FP} + V_{RC} \quad (68)$$

Building materials

$$M_C = C \times V_{CB} \quad (69)$$

$$V_G = G \times V_{CB} \quad (70)$$

$$V_S = S \times V_{CB} \quad (71)$$

where, $C = 325$ (72)

$$G = 0.8 \quad (73)$$

$$S = 0.4 \quad (74)$$

The different types of iron rods (NØD/m, where N is the number of iron rods per meter length, and D is the diameter of the iron rod) used to make such concrete bases are 6Ø6/m and 6Ø8/m:

$$N_{IL} = [(N_{IML} \times W_{CB}) + 1] \times 1.05 \times L_{CB} \quad (75)$$

$$N_{IW} = [(N_{IML} \times L_{CB}) + 1] \times 1.05 \times W_{CB} \quad (76)$$

$$N_{tIG} = N_{IL} + N_{IW} \quad (77)$$

$$N_{tI} = N_G \times N_{tIG} \quad (78)$$

$$N_{tSI} = \frac{N_{tI}}{L_{SI}} \quad (79)$$

$$M_I = N_{tSI} \times M_{IML} \times L_{SI} \quad (80)$$

where, $L_{SI} = 12$ (81)

if $N\phi D/m = 6\phi 6/m$, then $M_{IML} = 0.666$ (82)

if $N\phi D/m = 6\phi 8/m$, then $M_{IML} = 0.888$ (83)

The factor 1.05 is used to consider the interference between the iron rods. The standard iron rods are cut to shorter iron rods with a length of 1 m, they are then used to build up the concrete base.

Costs calculation

$$P_{tC} = P_C \times M_C \quad (84)$$

$$P_{tG} = P_G \times V_G \quad (85)$$

$$P_{tS} = P_S \times V_S \quad (86)$$

$$P_{tI} = P_I \times M_I \quad (87)$$

$$C_{tEC} = C_{EC} \times V_{CB} \quad (88)$$

$$C_{ICB} = P_{tC} + P_{tG} + P_{tS} + P_{tI} + C_{tEC} \quad (89)$$

$$C_{FCB} = \frac{C_{ICB}}{t_P} \quad (90)$$

$$C_{TCB} = C_{FCB} + C_{VCB} \quad (91)$$

where, $t_P = 20$ (92)

The Feeding Bunk(s) may be covered by a chemical material to prevent cow injuries. This operation may be carried out in a determined time interval. The costs of this operation are considered as part/whole value of C_{VCB}.

Results and Discussion

Spark maps

The spark map of the design model and the spark map of the concrete base model constitute the back diagram code of the software. The spark maps require some input data (Tables 1 and 2); however, the spark maps are empowered by a range of values for each required input data in order to help the designer/user in determining the required values. According to the inserted input data by the designer/user, the spark maps will compute the output data (Tables 3 and 4) using the mathematical models.

Table 1. Input data of the spark map of the design model.

Symbol	Description	Unit
N_{CF}	Number of Cows in Farm	
N_{CH}	Number of Cows in One House	
N_{CC}	Number of Cows in One Corral	
N_{CFP}	Cows per Feeding Place	
L_{FB}	Feeding Bunk Length	m/cow
A_{AC}	Allotted Area per Cow	m^2/cow
	Corrals Distribution (one or two sides of corrals, or one corral)	
W_{LB}	Width of Feeding Line & Feeding Bunk(s)	m
W_{FP}	Width of One Side of Feeding Places	m
W_{BB}	Width of Brisket Board	m
W_{RC}	Width of Rear Curb	m
L_{FP}	Length of One Side of Feeding Places in One Corral	m
W_{FP}	Width of One Side of Feeding Places	m
L_{SH}	Shade Length	m
S_F	Shade for Feeding	%
S_L	Shade for Laying	%
A_O	Office Area	m^2
A_H	Area of One House	m^2
A_{FS}	Forage Storage Area	m^2
A_{MC}	Area of Milking Center	m^2
A_M	Manure Lagoons/Tanks Area	m^2
	Construction Material (Steel or Concrete)	
	Cowshed Orientation (East-West, North-South, or Others)	
	Floor Material (Concrete, Sand, or Chopped Straw)	
	Roof Material (Reed, Straw, Aluminum, or Concrete)	
	Climate Conditions	

Table 2. Input data of the spark map of the concrete base model.

Symbol	Description	Unit
	Corrals Distribution (one or two sides of corrals, or one corral)	
L_{CB}	Concrete Base Length	m
W_{LB}	Width of Feeding Line & Feeding Bunks	m
W_{FP}	Width of One Side of Feeding Places	m
W_{RC}	Width of Rear Curb	m
W_{BB}	Width of Brisket Board	m
T_{LB}	Concrete Base Thickness (Feeding Line & Feeding Bunks)	m
T_{FP}	Concrete Base Thickness (Feeding Places)	m
H_{BB}	Height of Brisket Board	m
H_{RC}	Height of Rear Curb	m
	Type of Iron Rods (NØD/m: 6Ø6/m or 6Ø8/m)	
N_{IML}	Number of Iron Rods per One Meter Length of Concrete	
N_G	Number of Gridirons	
L_{SI}	Length of One Standard Iron Rod	m
M_{IML}	Mass of 1 m Long of Iron Rod	kg/m
P_G	Price of 1 m^3 Gravels	Currency/m^3
P_C	Price of 1 kg Cement	Currency/kg
P_S	Price of 1 m^3 Sand	Currency/m^3
C_{EC}	Employment Costs for 1 m^3 of Concrete	Currency/m^3
P_I	Price of One Ton of Iron Rods	Currency/Ton
t_P	Project Lifetime	Year
C_{VCB}	Variable Costs of Concrete Base	Currency/Year

Table 3. Output data of the spark map of the design model.

Symbol	Description	Unit
L_C	Corral Length	m
W_C	Corral Width	m
A_C	Corral Area	m^2
N_{HC}	Number of Corrals in One House	
W_{CB}	Concrete Base Width	m
A_H	Area of the House	m^2
W_H	House Width	m
L_H	House Length	m
H_C	Cowshed Height	m
A_{FP}	Area of One Side of Feeding Places in One Corral	m^2
R_A	Ratio of Feeding Area to Corral Area	
W_{SH}	Shade Width	m
A_{SHL}	Shade Area for Laying	m^2
A_{SHF}	Shade Area for Feeding	m^2
A_{SH}	Shade Area	m^2
N_{HF}	Number of Houses in Farm	
A_{tF}	Total Area of Farm Facilities	m^2

Table 4. Output data of the spark map of the concrete base model.

Symbol	Description	Unit
W_{CB}	Concrete Base Width	m
V_{FP}	Concrete Volume of the Feeding Places	m^3
V_{RC}	Concrete Volume of the Rear Curbs	m^3
V_{LB}	Concrete Volume of the Feeding Line & the Feeding Bunks	m^3
V_{BB}	Concrete Volume of the Brisket Boards	m^3
V_{CB}	Total Volume of the Concrete Base	m^3
V_G	Gravels Volume	m^3
M_C	Cement Mass	kg
V_S	Sand Volume	m^3
N_{IL}	Number of Iron Rods in Length	
N_{IW}	Number of Iron Rods in Width	
N_{tIG}	Total Number of Iron Rods in One Gridiron	
N_{tI}	Total Number of Iron Rods	
N_{tSI}	Total Number of Standard Iron Rods	
M_I	Iron Mass	Ton
P_{tI}	Total Price of Iron Rods	Currency
P_{tG}	Total Price of Gravels	Currency
P_{tC}	Total Price of Cement	Currency
P_{tS}	Total Price of Sand	Currency
C_{tEC}	Total Employment Costs of Concrete	Currency
C_{ICB}	Capital Investment of Concrete Base	Currency
C_{FCB}	Fixed Costs of Concrete Base	Currency/Year
C_{TCB}	Total Costs of Concrete Base	Currency/Year

Software

The developed software is able to plan and design dairy housing systems (corrals systems), specify corrals and house dimensions, and compute the required amounts of construction materials (iron rods, cement, sand, and gravels) to build the required concrete base. Furthermore, it calculates the capital investment and the fixed, variable, and total costs. However, 2 user interfaces (Figures 7 and 8) were developed in order to be used to insert the input data into the electronic spark maps. The output data of both spark maps will be shown in 2 separated windows (Figures 9 and 10).

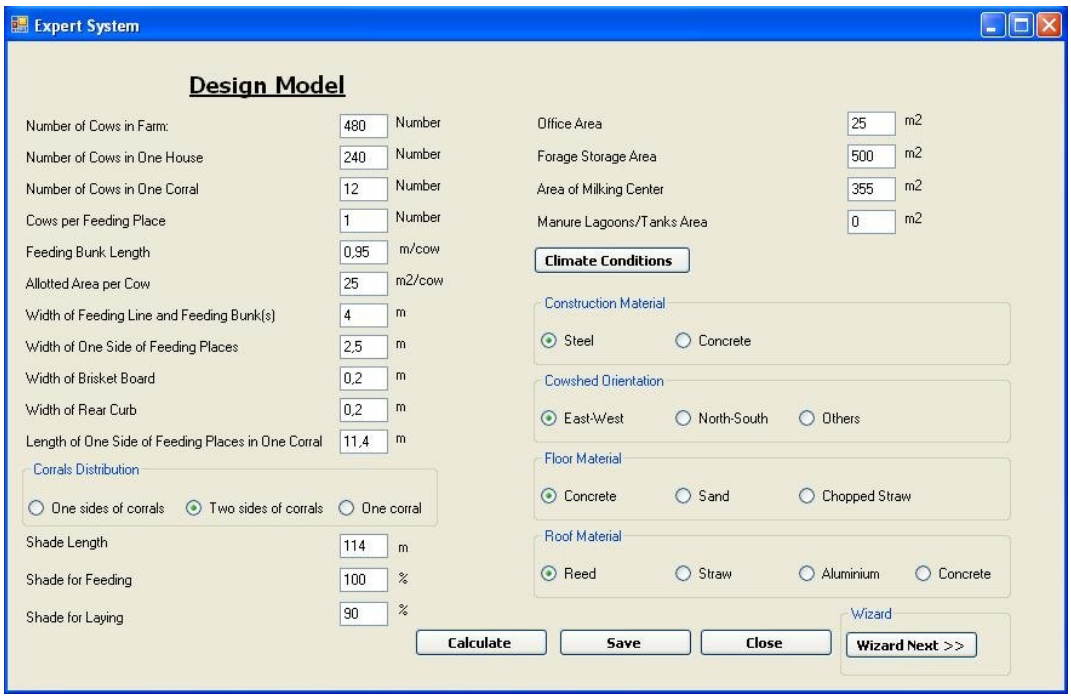

Figure 7. User interface of the input data of the design model.

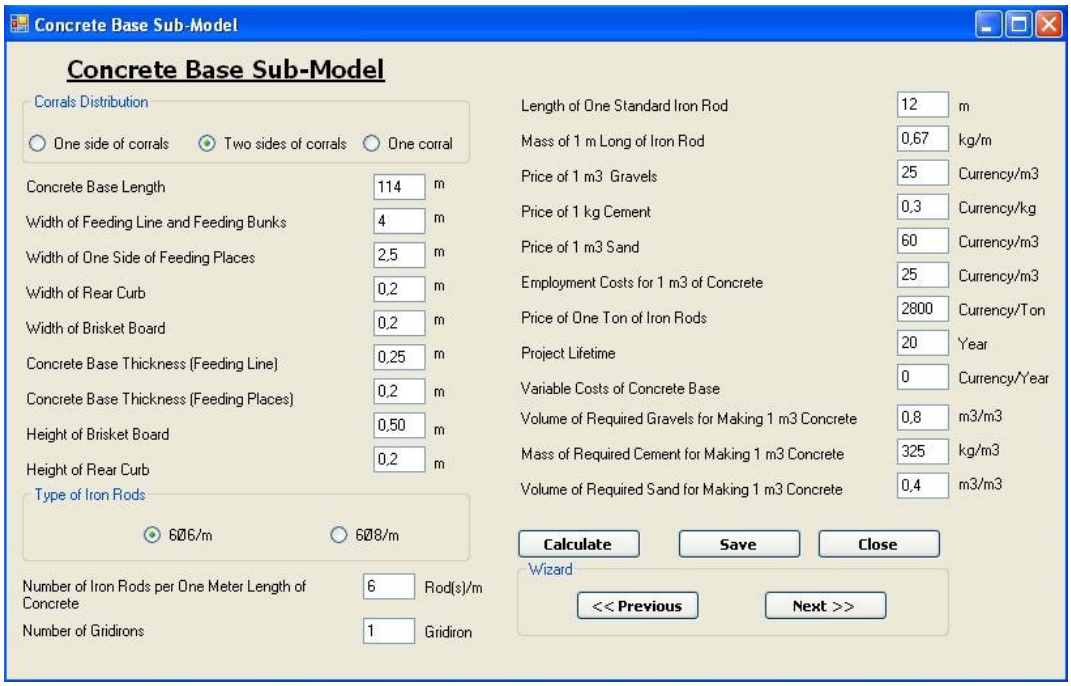

Figure 8. User interface of the input data of the concrete base model.

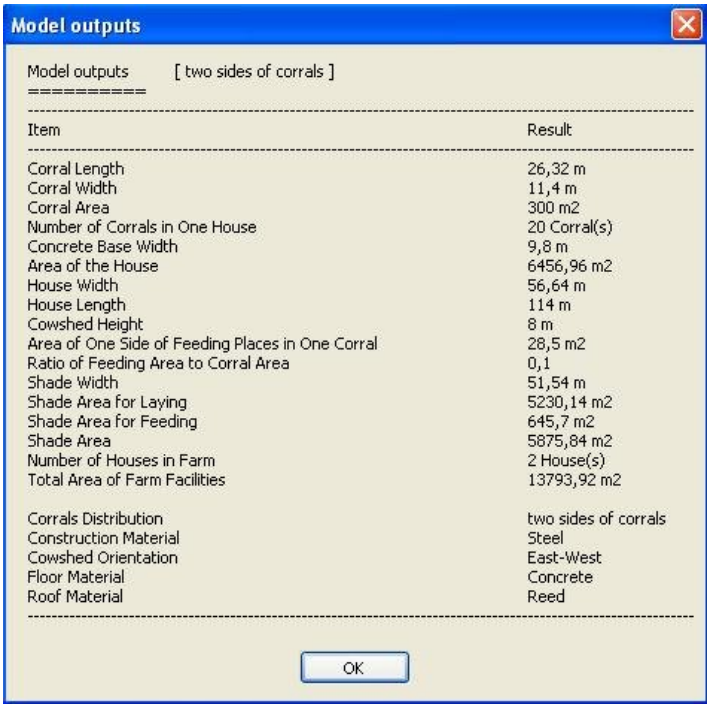

Figure 9. User interface of the output data of the design model.

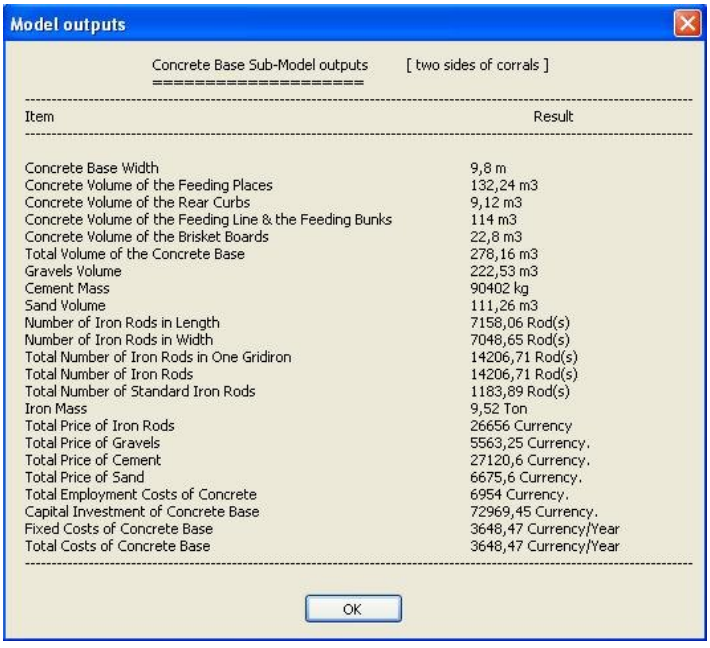

Figure 10. User interface of the output data of the concrete base model.

Model validation

Data of 6 Egyptian dairy farms were used to perform the model validation and the software evaluation. The farms were: (1) El-Tobgy Farms which housed 480 cows and located in El-Fayoum; (2) Dina Farms -sector 1- which housed 600 cows and located at Cairo-Alexandria desert road; (3) Dina Farms -sector 2- which housed 700 cows and

located at Cairo-Alexandria desert road; and three other small dairy farms where their data were acquired from the Cattle Information System of Egypt (CISE). The statistical analysis of the actual and calculated values (Table 5) elucidated that the COVs were 2.90% (σ = 0.01), 5.54% (σ = 0.03), 4.12% (σ = 0.01), 7.31 % (σ = 0.13), and 3.59% (σ = 0.03) for the amounts of concrete, gravels, cement, sand, and iron rods, respectively.

Safety emphasis

Unlike the traditional methods therewith making mistakes is possible, using this software diminishes the possibility of making mistakes and, therefore, improving safety. Further, this paper provides a new tool for planning and designing dairy housing in hot climates, where using a preset tool enhances safety measures. On the other hand, this tool is validated using actual data in order to assure high safety levels and to uproot system errors.

Future developments

For future developments, it would be wise to undertake agro-ecological evaluation of the different housing systems developed in this study with the methodology developed by Albino and Callado (2012). The main objective is to evaluate the environmental impact of these housing systems using the "Emergy Analysis". For this purpose, input data as materials, services, natural renewable/nonrenewable sources are required, where the analysis will be based on energy flows, transforming all inputs and outputs in a common unit.

Conclusions

Two mathematical models were developed to plan and design dairy housing systems (corrals systems) and the required concrete constructions. Subsequently, an electronic spark map (decision tree) was developed for each mathematical model, and then the mathematical models were integrated into the electronic spark maps. Afterwards, C# programming language was used to develop the software by integrating the electronic spark maps into one software program, using the spark maps to form the back diagram code of the software, and making the user interface to ease the use of the program. This method represents a new approach for developing software programs by using the mathematical models for practical implementation. The software is able to plan and design corrals systems, specify corrals and house dimensions, and compute the required amounts of construction materials (iron rods, cement, sand, and gravels) to build the required concrete base. Furthermore, it calculates the capital investment and the fixed, variable, and total costs of the constructions. Data of 6 dairy farms were used to validate the model, and to evaluate the software. The coefficients of variation (COVs) range between 3% and 7%. Further research to be suggested, is to implement similar methodology for developing software for planning and designing several dairy farm facilities (milking parlor, forage storage etc.,). This further research is a part of our project that next step is to develop software for the aforementioned farm facilities.

Table 5. Model validation.

	Parameter	L_C	W_C	N_{HC}	W_{CB}	V_{CB}	V_G	M_C	V_S	M_I
Farm 1	Actual Value	26.15	11.52	20	9.80	282.50	225.98	91415	114.50	9.64
	Calculated Value	26.32	11.40	20	9.80	278.16	222.53	90402	111.26	9.52
Farm 2	Actual Value	22.50	9.00	20	9.60	194.06	155.19	62785	78.50	7.38
	Calculated Value	22.22	9.00	20	9.60	193.05	154.44	62741	77.22	7.32
Farm 3	Actual Value	21.50	20.50	1	4.70	23.52	18.82	7609	9.52	1.06
	Calculated Value	22.00	20.00	1	4.70	23.40	18.72	7605	9.36	1.05
Farm 4	Actual Value	23.50	17.00	1	4.20	24.87	19.91	8049	10.07	1.00
	Calculated Value	23.53	17.00	1	4.20	24.75	19.80	8043	9.90	0.99
Farm 5	Actual Value	35.50	14.20	1	3.65	27.16	21.74	8789	11.03	1.22
	Calculated Value	35.29	14.17	1	3.65	27.03	21.62	8783	10.81	1.21
Farm 6	Actual Value	28.10	9.60	6	6.90	95.97	76.79	31060	38.90	3.41
	Calculated Value	28.13	9.60	6	6.90	95.50	76.40	31037	38.20	3.38

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